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TWO FUNGI CAUSING LEAF SPOT OF PEANUT [1]

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INTRODUCTION

The commonly cultivated varieties of peanut (*Arachis hypogaea* L.), especially the Spanish varieties, are seriously defoliated during the summer and early fall by two *Cercospora* leaf spots. Both leaf spots occur regularly on peanuts throughout the peanut-growing areas of the United States and apparently are destructive wherever peanuts are grown (7),[3] often making the crop unfit for hay and undoubtedly bringing about marked decreases in yield of pods.

Although several different species of fungi have been described from various parts of the world as causing leaf spots of peanuts, Woodroof (7) has shown by an extensive review of the literature and a study of many exsiccati that *Cercospora arachidicola* Hori and *C. personata* (B. and C.) Ell. and Ev. are the only valid species that have been described. She has likewise shown that both fungi cause destructive leaf spotting of peanuts in Georgia, whereas these diseases in the United States were formerly attributed to *C. personata* alone.

The present work is a report of the results of an investigation of the life history and cytology of the two peanut leaf spot fungi which the writer has had under way since 1934.

MYCOSPHAERELLA ARACHIDICOLA N. SP.

THE DISEASE

While all parts of the plant are subject to attack, symptoms on the leaflets are more striking and, perhaps, more destructive. When the leaf spots are first noticeable, they appear as slightly paler areas on the upper surface of the leaflets, as though the chlorophyll was fading, or blanching, slightly. Careful observation of the lower surface at this time indicates the presence of small areas in which the epidermal cells have collapsed and lost connection with the underlying mesophyll. Following this, the lesion develops fairly rapidly, taking on a distinct yellowish hue on the upper surface of the leaflet. Often a very small necrotic area becomes evident in the center of the developing lesion, before or during the time the yellow discoloration is developing. At maturity, the leaf spots appear as distinct necrotic areas, circular to irregular in outline, and often coalesce. They range in diameter from 1 mm to 1 cm or more, and are almost always surrounded by a yellow halo of varying width which blends gradually

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[3] Reference is made by number (italic) to Literature Cited, p. 332.

into the green of the leaf on the outer edges (fig. 1, *C*, *D*). On the upper surface, the necrotic areas vary from reddish brown to black, while on the lower surface they generally appear in lighter shades of

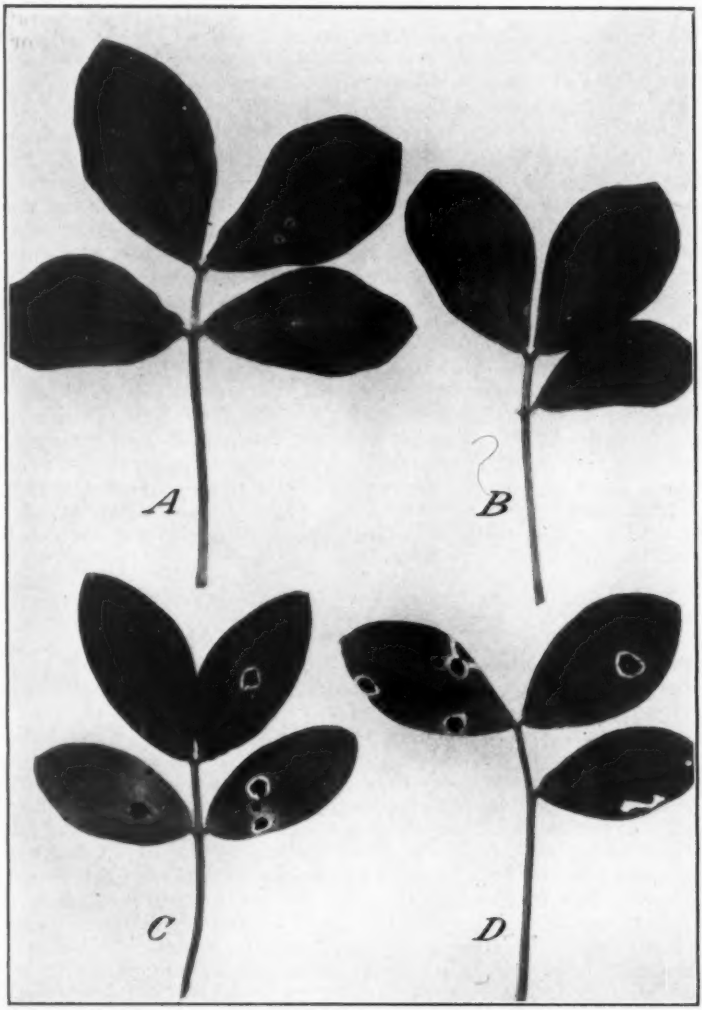


FIGURE 1.—*A* and *B*, Peanut leaflet inoculated in the greenhouse with conidia from ascospore cultures of *Mycosphaerella arachidicola*. Photograph taken 3 weeks after inoculation. Only the terminal leaflets were inoculated. *C* and *D*, Typical lesions of *M. arachidicola* on peanut leaflets from field collections.

brown to tan. The halos are much less distinct or even indistinguishable on the lower surface. Observations tend to show that the halo surrounding each lesion is perhaps in some way related to the carbo-

hydrate content of the leaf cells, since they appear best developed on leaves supposedly high in carbohydrates.

Evidence secured from many conidial inoculations indicates that infection is accomplished through either leaf surface, though the inoculum adheres best on the lower surface, and that penetration occurs directly through the lateral faces of the epidermal cells or by way of open stomata (fig. 4, *A*). Sections of young lesions show the mycelium of the parasite to be first intercellular, but the host cells are killed rapidly somewhat in advance of the mycelium, and the mycelium then penetrates the walls of the dead cells. No evidence of haustoria could be found. Conidiophores are at first developed only on the upper surface of the lesions, but often become amphigenous with age. They develop from subepidermal or subcuticular hyphae, which usually continue growth and produce more or less definite stromatic bases. The conidiophores quickly become yellowish brown and as conidia are formed and abjointed apically, the conidiophores continue to grow past them, and thus become geniculate. The conidia are usually attached at the broad end and leave definite scars on the conidiophores as they are abjointed and fall away.

The conidia are colorless to pale olivaceous in color, obclavate, often considerably curved, and measure from 37μ–108μ × 2.7μ–5.4μ. The length, and particularly the number of septations, are influenced by weather conditions at the time of conidial production. The number of septations varies from 3 to 12; shorter spores and fewer septations being associated with dry weather.

Conidia have been observed to germinate within 3 to 8 hours when moisture, oxygen, and temperature conditions were ideal. Germ tubes emerge from the terminal cell at either or both ends of the spore, and often from other cells as well. When completely covered by water, so that the oxygen supply is diminished, the spores rarely germinate at all, but often the cells become distorted through swelling. This condition is likewise evident when insufficient moisture is present. Abundant germination was always secured when the spores were placed on the surface of 2-percent agar at room temperature.

SPERMOGONIA

Spermogonia have been reported for this fungus on several occasions, both from field collections (*6*, *7*) and from studies on herbarium material. No record appears in the literature, however, of any studies on their development and structure. Spermogonia begin to appear on fallen leaflets at Experiment, Ga., during the latter part of September or early in October and continue to develop throughout the fall and winter. Occasionally young spermogonia are found as late in the spring as March, but this is rare. They are developed on either leaf surface, but are more often epiphyllous. They originate either within old conidial stromata or within separate stromata that develop after the death of the leaflets.

It is difficult to identify the earlier phases of spermogonial development, but in those cases that are certain the fundaments arise either subcuticularly or subepidermally, by the continued branching and interlacing of hyphae, which soon form compact globose to somewhat oval-shaped masses of stroma that continue to become larger and more complex until the cuticle or epidermis is ruptured (pl. 1, *A*).

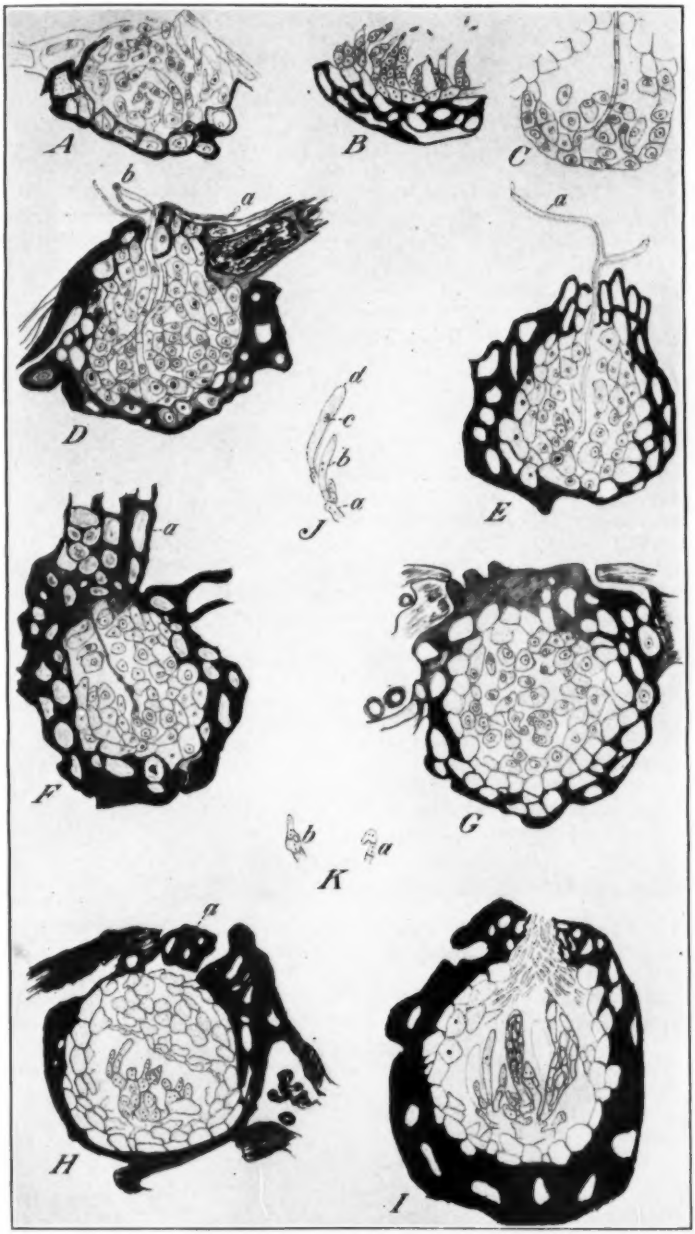
As development progresses, cells toward the center of the young spermogonium enlarge, the nucleus of each cell divides twice, and ultimately four uninucleate spermatia are formed in each cell after the fashion first described by Higgins (*3, pt. I*) and later by Jenkins (*5*) (pl. 1, *B*). The spermatia usually lie in tetrads but often appear in linear arrangement, and are liberated one at a time through a sterigma on each spermatial mother cell. After they have discharged their spermatia, the mother cells disintegrate and in so doing create enough bulk and pressure to force the spermatia out of the spermogonium. At maturity, the cavity is filled with spermatia and disrupted mother cells, surrounded by a single layer of spermatiferous cells, which is in turn enclosed by the spermogonial wall. Mature spermatia are rod-shaped, 1.5μ–$3\mu \times 0.5\mu$–1μ, and each possesses a deeply staining, centrally located, nucleus. Spermogonia which develop within conidiophore bases seem to have a different initial history. The pseudoparenchymatous cells toward the center of the stromatic mass are influenced to renewed activity, as evidenced by their staining reactions, and the cells lose their pseudoparenchymatous relationship. From this point onward, development is identical with the above-described process.

PERITHECIA

During early development, perithecia are practically identical with spermogonia as regards time, location in the leaf tissue, and mode of origin. They differ markedly, however, in that the cells toward the center of the young perithecium do not become enlarged as in the young spermogonium. Rather early in the development of the perithecium, one to several deeply staining archicarps, each with a somewhat enlarged, uninucleate basal cell and a long, several-celled trichogyne, are produced (pl. 1, *C*, *D*). The nuclei of the basal cells are very prominent, while those of the trichogynes are somewhat smaller and stain somewhat less distinctly. It is extremely difficult to identify the very young archicarps, but in those cases in which the writer could be certain, they appeared to arise as specialized branches from the plectenchymatous hyphae at or near the base of the young perithecium. The trichogynes often weave intricately among the sterile hyphae of the young perithecium and emerge to coil (pl. 1, *D*) or ramify (pl. 1, *E*) along the surface of the leaf. In certain preparations (pl. 1, *E*) the trichogyne is not only branched, but is apparently encased by a (chitinous?) sheath, except at the tip. This feature, if constant, may help to explain why the trichogynes are receptive only at the tips. The fact that the trichogynes often branch, an observation, which to the writer's knowledge, has not heretofore been reported

EXPLANATORY LEGEND FOR PLATE I

A, Young spermogonium of *Mycosphaerella arachidicola* in the upper surface of a leaflet, showing the enlargement of cells near the center; *B*, Portion of a mature spermogonium showing origin of spermatia; *C*, Young perithecium showing origin of the archicarp; *D*, Young perithecium containing two archicarps (note the attached, empty spermatium (*a*) and the sperm nucleus within the coiled trichogyne (*b*)); *E*, Young perithecium with a branched trichogyne. The exposed parts are covered by a (chitinous?) layer except at the tip (*a*); the sperm nucleus has reached the base of the trichogyne; and the protoplasm of the trichogyne is disorganized; *F*, Young perithecium developing in the base of a conidiophore fascicle (*a*), showing the sperm nucleus within the basal cell of the archicarp; *G*, An older perithecium. The ascogonium contains four pairs of nuclei; *H*, Early development of ascogenous hyphae. The paired nuclei within the branched ascogonium are embedded in dense masses of cytoplasm. The stromatic plug (*a*) is being pushed out in the formation of the ostiolum: *I*, Mature perithecium, showing ascogenous hyphae, young asci, mature asci, and periphyses; *J*, Ascogenous hypha (*a*) young ascus prior to fusion of nuclei (*b*); young ascus with the primary ascus nucleus (*c*); and a hyaline area at the apex of the ascus (*d*); *K*, beginning of crozier formation (*a*), and the process completed (*b*). All sketches drawn to scale with the aid of a camera lucida. × 1,320.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

in this genus of fungi, raises interesting speculations as to phylogenetic relationships.

SPERMATIZATION

Spermatia become attached to the tip or very near the tip of the trichogyne. Higgins (*3, pt. III*) was able to distinguish a receptive papilla on the trichogyne in *Mycosphaerella tulipiferae*. Though this feature could not be distinguished in the present work, its presence is suspected. The actual passage of the sperm nucleus from the spermatium into the trichogyne was not observed. Observations on many preparations, some showing attached, nucleated spermatia, and others showing attached, empty spermatia, together with the fact that attached, empty spermatia are consistently correlated with the presence of an extra, readily distinguishable nucleus in the trichogyne, have convinced the writer that spermatization does occur regularly (pl. 1, *D*, *E*). It is significant to note, also, as first described by Higgins (*3, pt. III*), that the nucleus and cytoplasm of the trichogyne disintegrate progressively as the sperm nucleus migrates down the trichogyne. In the present work, the presence of a multicellular archicarp makes this process all the more convincing (pl. 1, *C*, *D*, *E*). The writer believes that a more critical study of this phase of development would demonstrate the actual passage of the sperm nucleus into the trichogyne. The sperm nucleus gradually enlarges as it migrates down the trichogyne toward the nucleus in the basal cell. Shortly after it reaches the basal cell, it is indistinguishable from the basal nucleus. No fusion takes place between the sperm and basal nuclei. Instead, the ascogonium enlarges, and as it does so, the nuclei divide conjugately.

MATURATION

Several to many pairs of nuclei are formed in the enlarged ascogonium before ascogenous hyphae become evident (pl. 1, *G*). Shortly before and during the time ascogenous hyphae are being formed, the enlarging ascogonium becomes variously branched and contorted (pl. 1, *H*). At this time denser masses of cytoplasm enclose the paired nuclei (pl. 1, *H*), in this respect resembling the situation found in *Mycosphaerella tulipiferae* (*3, pt. III*). Initial ascogenous hyphae (pl. 1, *H*, *I*) are scarcely distinguishable from ascogonial branches, but later, when crozier formation begins (pl. 1, *K*), those hyphae that will form asci are readily distinguishable. Crozier formation differs in no important respects from that described by Higgins (*3, pt. III*). As the young ascus begins to elongate, the paired nuclei fuse to form the primary ascus nucleus. This nucleus enlarges somewhat (pl. 1, *J*, *c*) and begins a series of three divisions, resulting in the production of eight nuclei. Single-celled spores are formed by the condensation of cytoplasm about each nucleus. Soon the nucleus of each young spore divides once, and cross septa are formed which divide each spore into two-celled, biseriately arranged spores, the upper cell usually being somewhat thicker and shorter than the lower. At the same time, the ascogenous hyphae directly beneath and the thin-walled parenchyma cells above the developing asci are crushed by the pressure of the elongating asci (pl. 1, *H*).

During ascospore formation, the cytoplasm remains uniformly more hyaline at the apex of the ascus. It is in this area that the bitunicate nature of the ascus first becomes distinguishable (pl. 1, *I*, and *J*, *d*).

Not all the asci of a given perithecium mature at the same time. Almost without exception, practically all phases of ascus development were found in perithecia which were actively discharging mature spores (pl. 1, *I*).

As the first ascospores begin to mature, certain cells near the apex of the perithecium begin to multiply, and as this area is cracked open by the stress of unequal growth pressure (pl. 1, *H*, *a*) these cells line the ostiolum with periphyses (pl. 1, *I*). The periphyses are destroyed by the asci as they elongate and push through the ostiolum during spore discharge.

Several factors influence perithecial formation in nature. One of the limiting factors at Experiment seems to be sufficient rainfall during spermogonial discharges. During the several seasons the writer has pursued this study, it has been shown conclusively that unless the overwintering leaflets are wet during the period in which spermatia are being discharged, no perithecia are formed. The experiment was outlined in three parts, one, in which the leaves were sheltered and kept dry, another, in which the leaves were dependent on rains and dew for spermatization, and another in which the leaves were artificially sprinkled. Perithecia were produced only when the leaves were artificially sprinkled or when rain fell during spermatial discharge. Even under ideal conditions, perithecia are none too abundant, which may be explained in part by the fact that the leaflets, except for the lesions, disintegrate rapidly, thus affecting the nutrition of the overwintering fungus.

Temperature likewise seems to play an important part in perithecial production. Overwintering leaves were brought into the laboratory during February and March and placed in moist chambers. In each instance, mature perithecia and spores were secured in from 2 to 3 weeks, while mature perithecia and spores were never found in the field earlier than May 31. Spore discharge continued over a period of several weeks.

DEVELOPMENT IN CULTURE

Conidia germinate readily on agar under favorable conditions. Single- and multiple-spore isolations of conidia were obtained by streaking water suspensions of spores on tap-water agar in Petri dishes and transferring single or several spores to various types of agar in tubes. Satisfactory cultures were obtained on several agar media as malt, onion, synthetic, potato-dextrose, whole-milk, rice, oatmeal, corn-meal, peanut-leaf decoction, and on such other media as surface- and steam-sterilized peanut leaves and stems. On all these substrates, growth from single spores is visible to the unaided eye in about 3 days as whitish, sharply delimited, pulvinate colonies. Conidia are produced in each case in from 56 to 72 hours at room temperature (fig. 2, *C*). After 4 to 7 days cultures on all media assume an olivaceous color, and except in onion agar, on which conidial production is sustained for several days, conidial production ceases as the colonies begin to change color. Hard agars tend to keep the cultures from spreading. After 3 to 4 weeks the cultures on all these media have spread considerably and have begun to produce spermogonia in abundance. All attempts to produce the perfect stage in culture have failed. With the advent of spermogonia, or shortly before, the colonies become almost black and very stromatic.

Failure to obtain perithecia in culture was at first attributed to lack of free water and consequent failure of spermatization, but even cultures flooded with water and others flooded with suspensions of spermatia from different isolates, failed to produce perithecia, as did others grown in liquid cultures. Older cultures were fixed and sectioned in paraffin, but no evidence of archicarps could be found.

Isolations of ascospores were obtained by placing perithecial material below inverted tap-water agar plates and allowing the ascospores to shoot and stick to the surface of the agar. In this way patterns of eight spores, representing the content of individual asci, could be obtained absolutely free from contamination. Close or distant spacing of spores was obtained readily by raising or lowering the agar plates and, consequently, single- or multiple-spore isolations could be obtained at will.

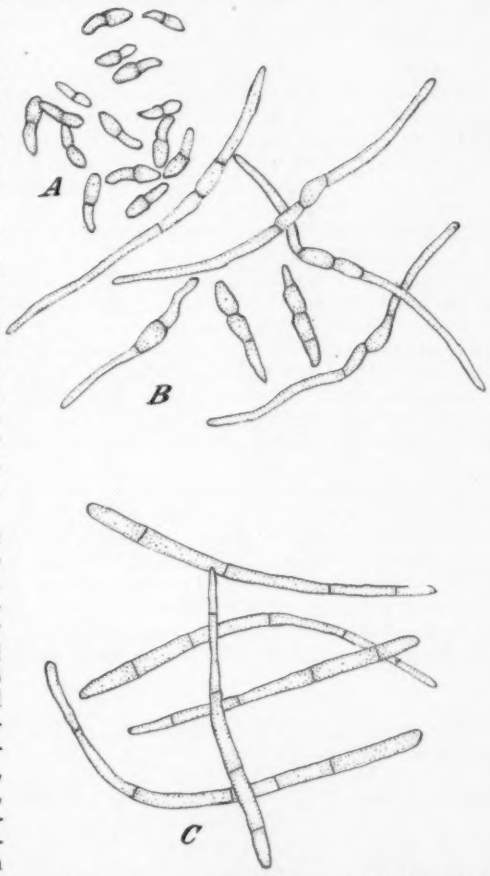


FIGURE 2.—*A*, Ascospores of *Mycosphaerella arachidicola*; *B*, germinating ascospores after 15 hours in tap water; *C*, Conidia from ascospore cultures. All × 750.

Ascospores germinate in from 3 to 4 hours under ideal conditions (fig. 2, *A*, *B*), but the resultant growth is scarcely visible to the unaided eye even after 5 to 6 days. Like the conidial cultures, ascospore cultures are whitish in color when first visible, but after 7 to 10 days, they, too, begin to assume an olivaceous color. Conidial production from ascospores in culture begins on any of the several media listed above after 5 to 6 days and, as before, is of rather short duration. In all respects, cultures from ascospores and from conidia are indistinguishable, except for the fact that ascospores produce a slower initial growth. Likewise, conidia produced on ascospore cultures (fig. 2, *C*) are indistinguishable from those produced in cultures from conidia and those produced on lesions on the peanut plant. After

about 3 weeks, both single and multiple ascospore cultures begin to produce spermogonia abundantly, but thus far all efforts to produce perithecia from ascospore cultures have failed.

GENETIC RELATION OF SPORE FORMS

Except for the fact that ascospores produced slower initial growth, cultures from conidia and from ascospores are identical in color, characteristics of growth, type of spores produced, and in all other measurable respects.

Since conidia are readily produced in artificial cultures, from both conidia and ascospores, these were used as the source of inoculum for inoculation tests, as well as conidia taken from peanut leaves. Healthy, growing peanut plants were selected for use in the inoculation tests, all of which were carried out in the greenhouse. Inoculum consisting of conidia in tap-water suspensions was placed on either upper or lower surface of leaflets, allowed to dry until barely moist, and then either wrapped with moist absorbent cotton and covered with cellophane or enclosed in bell glasses. Both methods proved successful and visible infections ranging from 50 to 100 percent were obtained in from 8 to 23 days. The checks remained free from infection. Under certain conditions of high temperature and low humidity, and when plants were low in carbohydrates, the percentage of infection was small and symptoms were slower in developing. The same was true when inoculations were made with conidia from ascospore cultures. Whether conidia from ascospore cultures, conidia from leaves, or conidia from conidial cultures were used, the symptoms produced were identical (fig. 1, *A*, *B*), the conidia borne later on the lesions were identical, and reisolations from these lesions were identical with the cultures from which the inoculum was taken.

Further evidence of genetic relation was found in numerous observations on sectioned material, in which definite hyphal connections could be demonstrated between spermogonia and perithecia, in which perithecia and spermogonia developed in conidiophore bases (pl. 1, *F*, *G*), and in which conidia were often produced the following spring on conidiophores arising from the exposed walls of spermogonia.

TAXONOMY

The form and development of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the two-celled hyaline spores, are all clearly characteristic of the genus *Mycosphaerella* Johans. A careful review of the literature shows that no species of *Mycosphaerella* has heretofore been reported on peanut leaves, and since the peanut has no close relative in this country, there can be little doubt that this is an undescribed species. It is, therefore, designated *Mycosphaerella arachidicola* n. sp., with the following diagnosis:

Mycosphaerella arachidicola n. sp.

Syn.: *Cercospora arachidicola* Hori, 1917, Nishigahara Agr. Expt. Sta. Tokyo, Ann. Rept. pp. 26–27 (*4*).
Cercospora arachidis P. Henn. var. *macrospora* Maff., 1922, Riv. Patol. Veg. 12: 7–11.

Perithecia scattered, mostly along margins of lesions, amphigenous, partly embedded in host tissue, erumpent, ovate to nearly globose, 47.6μ–84μ×44.4μ–74μ, black, ostiolum slightly papillate; asci cylindrical club-shaped, short stipitate, fasciculate, 27μ–37.8μ×7μ–8.4μ, aparaphysate, bitunicate, eight-spored; spores

uniseriate to imperfectly biseriate in the ascus, bicellular, the upper cell somewhat larger, slightly curved, hyaline, 7μ–15.4μ×3μ–4μ (average 11.2μ×3.64μ).

Hab. In overwintered lesions produced by the conidial stage on leaflets of *Arachis hypogaea*, Experiment, Georgia, maturing during June and July.

Spermogonia: Scattered in and along margins of lesions produced by the conidial stage, ovate to globose, black, amphigenous but perhaps more often epiphyllous, embedded in leaf tissue but later erumpent, ostiolate, 45μ–75μ×30μ–75μ (average 62.25μ×48.5μ); spermatia small, rod-shaped, hyaline, 1.5μ–3μ×0.5μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

On recently fallen leaflets, maturing throughout period from October through February.

Conidial stage: Spots irregularly circular, often confluent, varying in size, 1 mm to several centimeters, pale to dark brown, surrounded on upper surface by distinct yellow halo, mostly on leaflets but also on stems late in season; conidiophores mostly epiphyllous, sometimes amphigenous when old, arising from a stroma, fasciculate, geniculate, yellowish brown, continuous to one to several septate, 20μ–45μ×3μ–6μ; conidia colorless to slightly olivaceous, obclavate to clavate, often curved, 35μ–108μ×2μ–5.4μ, 4 to 12 septate, length and septation influenced by dry weather.

Conidial stage parasitic on all above-ground parts of *Arachis hypogaea*, but mostly causing leaf spots.

Perithecii sparsis, plerumque maculis marginatis, amphigenis, semi-immersis, punctiformibus, ovatis vel globosis, 47.6μ–84μ×44.4μ–74μ, nigris; ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, aparaphysatis, bitunicatis, octosporis, 27μ–37.8μ×7μ–8.4μ; sporidiis uniseriatis vel biseriatis, bicellularibus, cellula superiore leniter latiore, vix curvatis, hyalinis, 7μ–15.4μ×3μ–4μ, plerumque 11.2μ×3.6μ.

Hab. in foliis dejectis *Arachidis hypogeae*.

Spermogoniis autumno efformatis, sparsis, plerumque maculis marginatis, ovatis vel globosis, nigris, amphigenis plerumque epiphyllis, innato-erumpentibus, punctiformibus, 45μ–75μ×30μ–75μ, plerumque 62.25μ×48.5μ, spermatiis bacillaribus, hyalinis, 1.5μ–3μ×0.5μ–1μ.

Hab. in foliis dejectis *Arachidis hypogeae*.

Statu conidico in maculis orbicularibus v. irregularibus, confluentibus, magnis, ocraceis vel rubro-ferrugineis, ocraceis marginatis, plerumque foliis efformato; hyphis fertilibus plerumque epiphyllis, rare amphigenis, a stromate orientibus, fasciculatis, geniculatis, ochreis-ferrugineis, continuiis vel pluriseptatis, 20μ–45μ×3μ–6μ; conidiis hyalinis vel dilute olivaceis, obclavatis vel clavatis, plerumque curvulis, 35μ–108μ×2μ–5.4μ, 4–12 septatis.

Hab. in foliis et ramis vivis *Arachidis hypogeae*.

MYCOSPHAERELLA BERKELEYII, n. sp.

THE DISEASE

The disease produced by this fungus, like that produced by *Mycosphaerella arachidicola*, is evident on all parts of the plant above the soil. Late in the season it produces lesions on the stems, but it is predominantly a leaf spot disease. Early symptoms are indistinguishable from those caused by *M. arachidicola*, but very soon certain marked changes occur which make identification certain.

Perhaps the best diagnostic symptoms of *Mycosphaerella berkeleyii* leaf spot is the fact that the lesions are more circular than those of *M. arachidicola*, and the necrotic portions of the lesions, on both leaf surfaces, very early assume a very dark-brown to almost black color. At the same time, yellow halos are present on the upper surface of leaflets only on the more mature spots, this being in contrast to the very early-forming halos of *M. arachidicola*. Identification becomes unmistakable at or shortly before conidial production. At this time, the conidiophores are confined practically entirely to the lower surface of the leaflets and appear as definitely raised, dark-brown, stromatic

tufts, usually arranged concentrically and easily visible to the unaided eye (fig. 3). Later, the conidiophores may become somewhat amphigenous on old lesions, but even so the dark-brown, stromatic, concentrically arranged tufts of conidiophores could not be easily confused with the evenly distributed, olivaceous, more effuse conidiophores of *M. arachidicola*.

Inoculation tests with conidia indicate that the germ tubes of *Mycosphaerella berkeleyii* may enter either surface of the leaflet, through the lateral faces of epidermal cells or through open stomata, as do those of *M. arachidicola*. Sections of young and old lesions, however, show striking differences as regards the parasite-host relationships of the two fungi. The penetration hyphae of *M. berkeleyii* soon establish themselves intercellularly and remain so, sending variously branched to botryose haustoria into the cells of all leaf

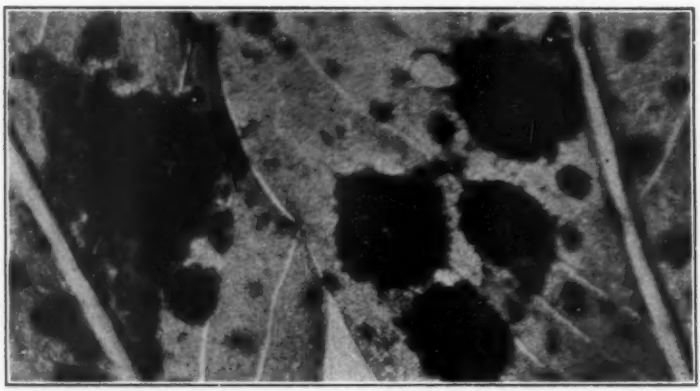


FIGURE 3.—Typical lesions of *Mycosphaerella berkeleyii*, on lower surface of peanut leaflets, from field collection. × 5.

tissues (fig. 4, *B*). The cells of the host are not killed in advance of the parasite. In fact, many preparations show haustoria established in apparently normal cells.

The conidiophores developed subcuticularly or subepidermally, and continue to become more stromatic even after they emerge through the leaf surface. At the time of conidial production, the conidiophores are coarse, reddish brown in color, except for hyaline tips, continuous to several septate, geniculate, and scarred by the abjointing conidia. The bases of the conidiophores are very stromatic, being composed of pseudoparenchyma.

The conidia are somewhat obclavate, but more generally cylindrical, except for somewhat attenuated tips, one to eight septate, pale brown to dilutely olivaceous, and measure 18μ–$60\mu \times 5\mu$–11μ. These conidia vary considerably under different conditions of moisture at the time they are formed, as do those of *Mycosphaerella arachidicola*.

SPERMOGONIA

Spermogonia have been reported for this fungus (*7*), but as in the case of *Mycosphaerella arachidicola*, no studies of their development

have been made. The cytological details of the development of the spermogonia and perithecia have not been fully worked out by the present writer, but it is hoped that this work may be completed at an early date. It was thought best to present here the work so far completed since the two organisms under consideration have been the subject of taxonomic confusion in the past, and since

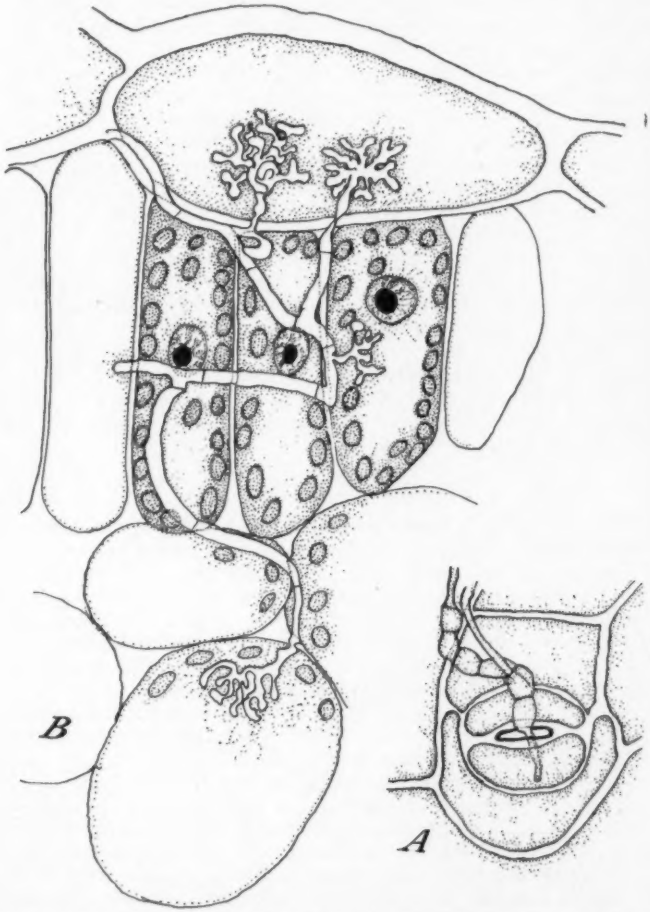


FIGURE 4.—*A*, Germ tube of conidium of *Mycosphaerella arachidicola* entering a stoma, × 750; *B*, section of a young lesion of *M. berkeleyii*, showing haustoria in epidermal and mesophyll cells, × 1320.

enough is known of their development to permit them to be definitely classified. The evidence so far obtained indicates that the spermogonia of this fungus originate and develop in a manner similar to those of *Mycosphaerella arachidicola*, and at comparable times of the year. The minor details of measurements, comparative amounts of stroma,

etc., while important, must await the completion of the cytological study.

PERITHECIA

Development of perithecia in this species appears to differ in several phases from that in *Mycosphaerella arachidicola*. Because of a greater amount of stroma in the old lesions, *M. berkeleyii* exhibits several initial variations which will require further study. Among these variations may be mentioned the fact that the archicarps are very numerous, often six to eight, in the young perithecia of *M. berkeleyii*. The large number of these structures and the fact that they interweave very intricately among other elements of the young perithecium, has slowed up this study considerably.

The work thus far indicates that spermatization is as essential to perithecial development in *Mycosphaerella berkeleyü* as in *M. arachidicola*. The same factors listed as influencing the perithecial development of *M. arachidicola* were found to influence perithecial development in *M. berkeleyii*. The experiments on the two fungi were planned and carried out concurrently. Although mature perithecia and spores were developed in moist chambers in the laboratory in March on material collected in February and March, mature perithecia and spores were not found in the field until early in July. This is an unusually late date for maturation of perithecia and spores of a species of *Mycosphaerella*, but, by allowing for a 2- to 3-week incubation period, the date of discharge of mature spores in the field is correlated very well with observations on time of initial infections on field-grown plants.

DEVELOPMENT IN CULTURE

Single and multiple spore isolations of conidia were obtained by the streak method outlined previously. In all cases, the same media were used as before mentioned. No difficulty was experienced in obtaining spore germination and conidial production in artificial culture. Given similarly ideal conditions, conidia of *Mycosphaerella berkeleyii* do not differ in percentage and mode of germination from those of *M. arachidicola*, except for the fact that the conidia of *M. berkeleyii* respond best to slightly lower temperatures.

On all substrata growth is extremely slow and becomes visible to the unaided eye only after 4 to 7 days. From the time the mycelium can be seen in culture, the hyphae are distinctly pigmented. At first the color is pale buff, but as the colonies age it becomes tawny or even reddish. Growth is always sparse. At first the aerial hyphae stand apart rather than form a compact growth as in *M. arachidicola*. Even when old, the colonies never spread much; instead, discrete tufts of tawny- to reddish-colored aerial hyphae develop from small stromatic bases. After 7 to 10 days or sometimes longer, while the colonies are still only a few millimeters in diameter, numerous spores are produced on typical conidiophores (fig. 5, *c*). In all respects, these spores are like those taken from peanut leaves, except, owing perhaps to the more constant moisture supply, they are uniformly longer. The writer tried on several occasions to cause the colonies to spread by jarring the cultures during spore production and thus knocking other spores onto the medium. In all cases colonies from these spores remained discrete from the original colony. After 3 to 4 weeks, the

colonies become stromatic, often decidedly reddish in color, and produce spermogonia in abundance. All attempts to produce the perfect stage in culture have resulted in failure; likewise no archicarps have ever been found in artificial cultures.

Ascospores isolated as described for *Mycosphaerella arachidicola* germinate in from 4 to 10 hours under laboratory conditions, but the resultant growth is scarcely visible to the unaided eye even after 7 to 8 days (fig. 5, *A*, *B*). In all respects, ascospore cultures are identical with conidial cultures, except for a slower initial growth, and produce typical conidia, after 7 to 12 days, identical in every way with those from conidial cultures. In fact, conidia are produced from ascospore cultures sometime before the colony is readily visible to the unaided eye, and the period of conidial production often extends over a period of a week or more. After 3 to 4 weeks, ascospore cultures likewise produce spermogonia in abundance, but all attempts to produce perithecia in culture have failed.

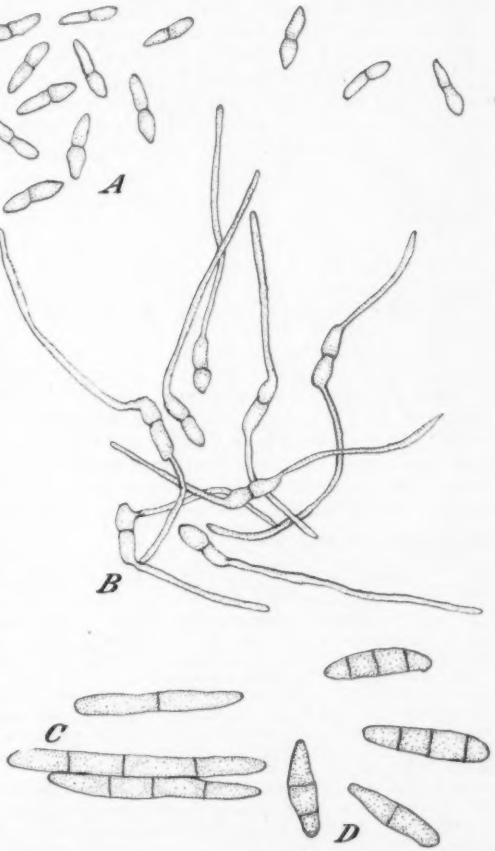


FIGURE 5.—*A*, Ascospores of *Mycosphaerella berkeleyii*; *B*, germinating ascospores after 15 hours in tap water; *C*, conidia from ascospore cultures; *D*, conidia from lesions obtained by inoculating peanut leaflets with conidia from ascospore cultures, 4 weeks after inoculation. All × 750.

GENETIC RELATION OF SPORE FORMS

Cultures and conidia produced on artificial media, from both ascospores and conidia, are identical in all measurable respects, except for the fact that the spores produced in culture are uniformly longer than those taken from peanut leaves and ascospores produce a slower initial growth than do conidia.

Tap-water suspensions of conidia, developed in culture from ascospores, were used as inoculum for inoculation tests, as well as conidia from conidial cultures and conidia from peanut leaves. Plants,

technique, and checks were as described earlier for *Mycosphaerella arachidicola*. The percentage of infection obtained was disappointingly low in those instances in which infection was obtained, but all lesions were identical with those on field material. The writer feels that these results may have been due to any one of several combinations of factors, including moisture, temperature, carbohydrate reserve of the plants used, as well as age of the plants. It is sufficient to say, however, that several inoculations produced typical infections, which yielded identical cultures when reisolated. The check remained free from infection. As a rule, infections were evident in from 10 days to 3 weeks, though some inoculations, in very hot weather, did not show symptoms of infection until after 4 weeks.

Further evidence of genetic relation was obtained from a study of sectioned material. In all cases thus far seen perithecia originate in the bases of conidiophore stromata, or are so close by that hyphal connections are perfectly evident. The lesion produced by the conidial stage is so characteristic and retains its identity so perfectly, that one can follow conidial, spermogonial, and perithecial development within the confines of lesions which are readily identifiable as being unmixed. This alone, in the absence of other data, would constitute weighty evidence of the genetic relation of these spore forms.

TAXONOMY

The form of the perithecia, the asci produced in fascicles, the absence of paraphyses, and the two-celled hyaline spores, are all clearly characteristic of the genus *Mycosphaerella* Johans. This species has apparently not been described previously. The writer proposes the designation *Mycosphaerella berkeleyii* in honor of the first author of this leaf spot fungus, with the following diagnosis:

Mycosphaerella berkeleyii sp. nov.

Syn.: *Cladosporium personatum* B. and C., 1875, Grev. 3: 106.
Cercospora personata (B. and C.) Ellis and Everhart, 1885, Jour. Mycol. 1: 63.
Septogloeum arachidis Rac., 1898, Ztschr. Pflanzenkrank. 8: 66–67.
Cercospora arachidis P. Henn., 1902, Hedwigia 41 (Beibl.): (15)–(18).

Perithecia scattered, mostly along margins of lesions produced by the conidial stage, amphigenous, partly embedded in host tissue, erumpent, broadly ovate to globose, 84μ–140μ×70μ–112μ, black, ostiolum slightly papillate; asci cylindrical club-shaped, short stipitate, fasciculate, 30μ–40μ×4μ–6μ, aparaphysate, bitunicate, eight-spored; spores uniseriate to imperfectly biseriate in the ascus, bicellular, the upper cell somewhat larger, slightly constricted at the septum, hyaline, 10.92μ–19.6μ×2.9μ–3.83μ (average 14.9μ×3.44μ).

Hab. In overwintered lesions produced by the conidial stage on leaflets of *Arachis hypogaea*, Experiment, Georgia, maturing during June and July.

Spermogonia: Scattered in and along borders of lesions produced by the conidial stage, ovate to globose, mostly heavy walls, black, amphigenous but perhaps more often epiphyllous, embedded in leaf tissue but later erumpent, ostiolate, 75μ–90μ×70μ–90μ; spermatia small, rod-shaped, hyaline, 1μ–3μ×0.5μ–1μ, arising endogenously, usually in fours within spermatiferous cells and liberated through sterigma-like processes.

On fallen leaflets, maturing throughout period from October through February.

Conidial stage: Spots mostly circular, often confluent, varying in size, 1 mm to several centimeters, brown to dark brown, often surrounded by a yellow halo on the upper surface when old, mostly on leaflets but often on stems late in season; conidiophores mostly hypophyllous, sometimes amphigenous when old, arising in more of less distinctly concentric tufts from heavy stromatic bases, fasciculate, geniculate, reddish-brown with mostly hyaline tips, continuous or one to several septate, 24μ–54μ×2μ–8.2μ; conidia somewhat obclavate but more generally cylindrical

with somewhat attenuated tips, pale brown to dilutely olivaceous, 18μ–60μ×5μ–11μ, one to eight septate, length and septations influenced by dry weather.

Parasitic on all above-ground parts of *Arachis hypogaea*, but mostly causing leaf spots.

Peritheciis sparsis, plerumque maculis marginatis, amphigenis, semiimmersis, punctiformibus, ovatis vel globosis, 84μ–140μ×70μ–112μ, nigris, ostiolis papillato praeditis; ascis cylindraceis clavatis, brevisme stipitatis, aparaphysatis, fasciculatis, bi-tunicatis, octosporis, 30μ–40μ×4μ–6μ; sporidiis uniseriatis vel biseriatis, bicellularibus, cellula superiore leniter latiore, leniter constrictis, hyalinis, 10.92μ–19.6μ×2.9μ–3.83μ plerumque 14.9μ×3.44μ.

Hab. in foliis dejectis *Arachidis hypogeae*.

Spermogoniis autumno efformatis, sparsis, plerumque maculis marginatis, ovatis vel globosis, nigris, amphigenis plerumque epiphyllis, innato-erumpentibus, punctiformibus, 75μ–90μ×70μ–90μ; spermatiis bacillaribus, hyalinis, 1μ–3μ×0.5μ–1μ.

Hab. in follis dejectis *Arachidis hypogeae*.

Statu conidico in maculis orbicularibus v. irregularibus, interdum confluentibus, magnis, brunneis vel atro-brunneis, plerumque ocraceis marginatis, plerumque foliis efformato; hyphis fertilibus hypophyllis, rare amphigenis, a stromate concentricis orientibus, fasciculatis, geniculatis, rubro-ferrugineis, sursum dilutioribus, continuis vel pluriseptatis, 24μ–54μ×2μ–8.2μ; conidiis sursum obclavatis plerumque cylindricis attenuatis apiciis, dilute brunneis vel dilute olivaceis, 18μ–60μ×5μ–11μ, one to eight septatis.

Hab. in foliis et ramis vivis *Arachidis hypogeae*.

For the convenience of plant pathologists and mycologists, type material of both species has been deposited in the following herbaria: Georgia Agricultural Experiment Station, Experiment, Ga.; Mycological Collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; Plant Pathology Department, Cornell University, Ithaca, N. Y.

DISCUSSION

The disease caused by *Mycosphaerella arachidicola* usually appears at Experiment early in July; that caused by *M. berkeleyii* usually appears a few weeks later. By allowing for an incubation period of from 2 to 3 weeks, the time of appearance of the diseases in the field is closely correlated with the dates the organsims begin discharging ascospores. Primary infections are rarely so numerous as to attract much notice.

During the years these diseases have been under observation, *M. arachidicola* has been for the most part more widespread than *Mycosphaerella berkeleyii*. It also reaches epiphytotic proportions during August and early September, whereas *M. berkeleyii* is most destructive from September through harvest. During the epiphytotic months, the nights are cool and humid, a condition which especially favors the rapid spread of these organisms. Though both leaf spots occur on a given plant or a given leaflet, and even mixed lesions (i. e., lesions in which both organisms are fruiting) are not uncommon in the field, *M. arachidicola* usually causes the most damage to Spanish peanuts in Georgia owing to the fact that these varieties are usually harvested before *M. berkeleyii* reaches epiphytotic proportions. On other varieties on which comparisons can be made, *M. berkeleyii* usually causes a more rapid defoliation than *M. arachidicola*.

SUMMARY

The morphology and life history of the two peanut leaf spot fungi known heretofore as *Cercospora arachidicola* Hori and *C. personata* (B. and C.) E. and E., which cause destructive leaf spots of *Arachi-*

hypogaea (peanut), have been studied over a period of three seasons. Each fungus was found to produce spermogonia and perithecia, in addition to conidia.

Apparently neither perithecial stage has heretofore been described, and the author accordingly proposes *Mycosphaerella arachidicola* sp. nov. for the perithecial stage of *Cercospora arachidicola* and *M. berkeleyii* sp. nov. for the perithecial stage of *C. personata*.

The development of spermogonia and perithecia of both fungi is initiated during early fall. The spermogonia mature and (usually) cease liberating spermatia by February, whereas the perithecia do not mature until late in the following spring.

Additional evidence is presented to indicate that spermatia function as male sexual elements in the production of perithecia.

The disease caused by *Mycosphaerella arachidicola* reaches epiphytotic proportions during August and early September, whereas that produced by *M. berkeleyii* is most destructive from September through harvest.

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HOPI COTTON, A VARIABLE SPECIES [1]

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INTRODUCTION

There is much interest at present in Hopi cotton (*Gossypium hopi* Lewton), a species formerly cultivated by the Hopi Indians of northeastern Arizona on the Hopi Indian Reservation. This interest, stimulated by tests conducted by the Bureau of Agricultural Economics of the United States Department of Agriculture,[2] has resulted in requests for seed from cotton breeders in this and foreign countries, who hope to combine the spinning qualities of the aboriginal cotton with the productivity of commercial varieties. Archeologists are interested in Hopi cotton on account of its probable identity with the fiber found in prehistoric ruins in the Southwest.[3]

BREEDING STOCK

The behavior of several strains of *Gossypium hopi* in the breeding plots at the United States Field Station, Sacaton, Ariz., indicates that the species is genetically heterozygous and capable of modification by selection. Until 1933 only two strains were grown at Sacaton. One of these, "Moqui," is an inbred descendant of seed collected in 1911 at Oraibi Village, on the Hopi Indian Reservation, by Frank A. Thackery.[4] The other, "Sacaton Aboriginal", descended from seed obtained from a Pima Indian at Sacaton, on the Gila River Indian Reservation. It was assumed to be the kind of cotton grown by the Pimas before the advent of the white man. As grown at Sacaton, the plants of both strains are erect, 5 to 8 feet high, and have pitted bolls and yellow pollen. They differ in these characters from Lewton's description and illustrations of *G. hopi*.

In 1932 C. J. King and George J. Harrison, of the United States Department of Agriculture, obtained seed cotton from an old Hopi Indian at Moencopi, on the Hopi Indian Reservation, Ariz., who told them that he was growing his third successive crop for ceremonial purposes. Replies to previous inquiries about the production of cotton on the Indian reservations had indicated that none had been grown for a number of years.

In 1933 several hundred hills of cotton from this new supply of seed, designated "Hopi Moencopi" or "Hopi M," were grown at Sacaton. Approximately 40 plants were selected for propagation, several of which showed different degrees of pitting of the surface of

[1] Received for publication July 9, 1937; issued April 1938.

[2] ANONYMOUS. NEW FACTS ON STRENGTH OF COTTON START BREEDING FOR FINE SHORT STAPLE. Mid-South Cotton News 13 (11): 8. 1936.
CATES, J. S. NEW STAPLE BREEDING TARGET. Country Gent. 106 (9): 21, 84–85, illus. 1936.

[3] JONES, V. H. A SUMMARY OF DATA ON ABORIGINAL COTTON OF THE SOUTHWEST. N. Mex. Univ. Bull. 296 (Anthropological Ser. v. 1, no. 5): 51–64. 1936.

[4] LEWTON, F. L. THE COTTON OF THE HOPI INDIANS; A NEW SPECIES OF GOSSYPIUM. Smithsn. Misc. Collect. 60, no. 6, Pub. 2146, 10 pp., illus. 1912. (See p. 7.)

the bolls. The progenies grown in 1934 from self-pollinated seed continued to show segregation in respect to boll surface, and further selections were made. In addition, plants with white corollas, and others with cream-colored pollen, were observed and selected. From these selections, progenies have been isolated that breed true for white corolla, yellow corolla, cream-colored pollen, yellow pollen, smooth boll surface (oil glands embedded and confined to an area near the sutures), and pitted boll surface. Figure 1 shows the range of pitting found in bolls of these progenies of the Hopi Moencopi strain.

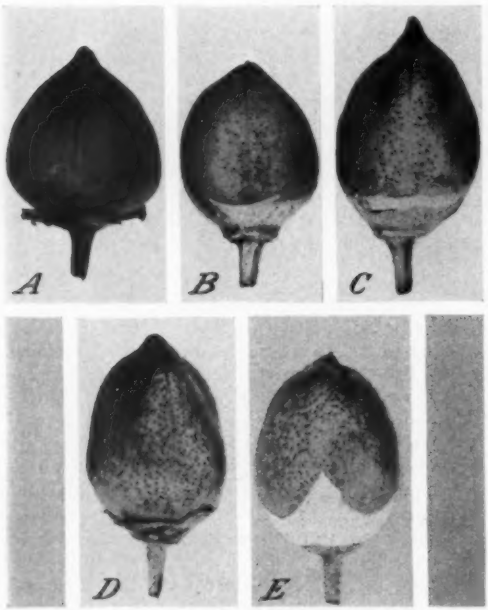

FIGURE 1.—Bolls from five progenies of the Moencopi strain of Hopi cotton showing differences in degree and character of the oil glands. The number of glands increases from none, or almost none, in *A* to numerous in *E*. In *A*, *B*, and *C* the glands, which are only along the sutures, are embedded, whereas in *D* and *E* they are in visible pits on the surface. Natural size.

In 1933 many of the plants exhibited the prostrate habit described by Lewton, having main stalks and limbs that reclined by the time they were 3 months old. In succeeding generations the progenies of plants from which self-pollinated seed was obtained tended to assume a more nearly erect habit. However, none of the plants in the progenies of the Moencopi strain have developed main stalks as strong as those of the Moqui and Sacaton aboriginal strains. This weakness of stems varied from progeny to progeny, being least pronounced in the progenies, grown in 1936, that were designated Hopi M 34–6–2 and M 34–9–2.

COMPARISON OF CHARACTERS

Table 1 presents a comparison of nine characters of the Hopi progenies grown at Sacaton in 1936. The first two progenies listed in the table, Hopi M 5–4–13 and M 5–4–10 (fig. 2), come nearest to matching Lewton's description, having yellow corollas, cream-colored pollen, and smooth boll surface. The next three selections from Hopi Moencopi have yellow instead of cream-colored pollen, and progenies M 34–6–2 and M 34–9–2 further depart from the original description by having the surface of the bolls conspicuously pitted like those of Pima (*Gossypium barbadense* L.). These two progenies approach the two long-inbred strains of Hopi—Moqui and Sacaton aboriginal—in plant habit, in corolla color, and in boll surface, and are the most productive

of the Moencopi strain. The last four progenies introduce another character, white corolla, and have boll surfaces intermediate in degree of pitting.

TABLE 1.—*Comparison of certain characters in progenies of Hopi cotton grown at the United States Field Station, Sacaton, Ariz., in 1936*

Progeny designation	Inbred generations	Corolla color	Pollen color	Boll surface grade[1]	Seed-cotton weight per boll	Seeds per boll	Seed index	Lint index	Lint percentage	Upper quartile fiber length
	No.				*Grams*	*No.*				*Inch*
Hopi M 5-4-13	3	Yellow	Light cream	0.15	1.63	16.1	8.05	2.00	20.0	0.93
Hopi M 5-4-10	3	do	Cream	.30	1.82	18.2	8.10	1.88	18.8	.96
Hopi M 6-10-1	3	do	Yellow	.94	1.26	12.6	7.70	2.26	22.8	.87
Hopi M 34-6-2	3	do	do	4.00	1.36	12.9	8.40	2.22	20.9	.98
Hopi M 34-9-2	3	do	do	4.00	1.40	13.8	7.90	2.18	21.9	.95
Moqui	13	do	do	3.75	1.95	16.5	10.04	1.78	15.2	.83
Sacaton aboriginal	14	do	do	3.75	2.04	20.0	8.62	1.54	15.6	.87
Hopi M 6-3-5	3	White	do	1.16	1.50	11.5	10.76	2.26	17.3	.88
Hopi M 6-14-1	3	do	do	1.96	1.79	15.6	9.00	2.48	21.6	.86
Hopi M 6-16-5	3	do	do	1.11	1.79	13.5	10.40	2.78	21.1	.84
Hopi M 6-16-7	3	do	do	1.34	1.65	13.2	9.98	2.56	20.5	.82

[1] Based on an arbitrary range of 5 grades, 0 to 4, inclusive, illustrated in fig. 1.

The undesirable character of Hopi cotton for commercial production is shown (table 1) by the seed-cotton weight per boll and the lint percentage. For the purpose of comparison, these and associated characters for Hopi and for Acala—the latter being the leading upland cotton variety grown in Arizona—are given in table 2.

TABLE 2.—*Comparison of certain characters in Hopi and Acala cottons*

Character		Hopi[1]	Acala
Seed-cotton weight per boll	grams	1.65	6.18
Seeds per boll	number	14.9	32.5
Seed index		9.0	13.0
Lint index		2.18	7.71
Lint percentage		19.6	36.0

[1] Means of the values for the respective characters listed in table 1.

In order to emphasize the small size of the Hopi bolls, it is computed that 1,403 would be required to furnish 1 pound of lint, as compared with only 204 bolls of the Acala variety. The values for seed index, lint index, and lint percentage show that the seeds of Hopi are not only small but are sparsely covered with lint.

The fiber length given in table 1 is the upper quartile of sorted arrays rather than the mean, as Webb [5] and his associates have found that in ginned cotton fiber the upper quartile length approximates the length determined by professional cotton classers. For fiber length Lewton [6] gives a range of 18 to 25 mm (0.70 to 0.98 inch). The Hopi progenies range from 0.82 to 0.98 of an inch in upper quartile length (table 1), and all of them seem to meet the requirements of the original description in having "white, strong, fine, and silky" lint.[6]

[5] WEBB, R. W. SUTER-WEBB COTTON FIBER DUPLEX SORTER . . . Amer. Soc. Testing Materials Proc. 35, v. 32, pt. 2, pp. 1-11, illus. 1932.
[6] LEWTON, F. L. See footnote 4, particularly p. 10 of reference.

Genetic experiments, involving interspecific crosses, confirm the evidence from morphological characters that *Gossypium hopi* is more closely related to *G. hirsutum* L. than to *G. barbadense*. Segregation in F_2 was much less pronounced in the cross with upland cotton (*G. hirsutum*) than in the cross with Egyptian cotton (*G. barbadense*),

FIGURE 2.—Plants of the Moencopi strain of Hopi cotton, progeny M 5-4-10, 107 days after planting. Note the well-developed fruiting branches. The plants were from 30 to 34 inches high.

and there was much less sterility in *hirsutum* × *hopi* F_2 than in *barbadense* × *hopi* F_2. A study of the behavior of several contrasting characters in intraspecific crosses among different progenies of *G. hopi* is now in progress.

SUMMARY

The data presented in this paper indicate that Hopi cotton, formerly grown by the southwestern Indians, is heterozygous and capable of modification by selection. Progenies have been isolated that breed true for each character of the following allelomorphs: (1) Yellow and white corollas, (2) yellow and cream-colored pollen, and (3) smooth and pitted bolls.

OXIDASE AND CATALASE ACTIVITY OF BARTLETT PEARS IN RELATION TO MATURITY AND STORAGE[1]

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INTRODUCTION

In a previous publication (*3*)[2] it was pointed out that Bartlett pears picked relatively immature gave a less desirable canned product than did those left on the tree until more mature. The less mature fruit tended to have a pale orange-yellow color in contrast to the clear translucent cream color of the more mature fruit. This difference in color was due to changes in the fruit itself and not to differences in processing. It was found that for best results Bartlett pears should be harvested at a pressure test of 17 to 15 pounds (as measured on the pared flesh by means of the United States Department of Agriculture pressure tester with the $\frac{5}{16}$-inch plunger) and stored immediately at 30° to 32° F. for 15 to 30 days. Longer periods in cold storage tended to exaggerate the undesirable color of the less mature fruit.

An oxidase is believed to be the principal agent responsible for the darkening of pears in preparation for canning. In commercial canneries the pared fruit is placed in a sodium chloride solution to inactivate the enzyme and prevent excessive oxidation until the fruit is ready for processing. If the oxidase is very active the darkening will be rapid and the fruit may be discolored before it can be placed in the brine solution, or the enzyme may not be completely inactivated and the darkening may continue while the fruit is being graded and placed in the can.

The work reported herein was undertaken in the hope that information might be obtained on the oxidase and catalase activity of Bartlett pears that would be of value in reducing the wide variations in color in the canned fruit. The investigations were made at Wenatchee, Wash., during the 1935 and 1936 seasons.

REVIEW OF LITERATURE

In the study of the physiological development and storage of fruits, catalase has been used more often than oxidase to indicate metabolic changes; consequently the literature dealing with catalase is much more voluminous. However, oxidase activity probably plays a more important part in the discoloration of fruit during canning operations and is therefore of greater interest from the standpoint of food processing.

Ajon (*1*), working with citrus fruits, reported that as the fruit ripened the oxidase gradually diminished from the center of the parenchyma outward to the periphery, finally almost, if not quite,

[1] Received for publication August 18, 1937; issued April 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 345.

disappearing. De Villiers (*2*) also reported a decrease in oxidase activity of the grape as the berry ripened. Catalase increased with maturity but decreased slightly with final stages of maturation.

Haber (*7*) found greater oxidase activity in ripe tomato fruits than in green ones; catalase activity, on the other hand, was greater in green mature fruits. Gustafson et al. (*5*) reported a slightly lower catalase activity in yellow-orange and orange-colored tomatoes than in orange-red and red ones.

Lüers et al. (*11*) found that barley increased in catalase content during ripening until it became yellow, after which it decreased while the resting state was attained. During storage there was no change in the catalase content. Noguchi (*13*) reported a gradual decrease, without any sudden change at any time, in the catalase content of rice stored for 18 months. The oxidase content was unchanged.

Neller (*12*) found that in apples free from break-down catalase tended to increase during the earlier, then to decrease during later periods of storage, corresponding to the youth and senescence of the fruit. Harding (*8*) found that under cold-storage conditions an increase in catalase activity of Grimes Golden apples was a fairly accurate index to the approach of soggy break-down. Overholser (*14*) found that catalase decreased in Bartlett pears as maturity advanced and that "the effect of the storage temperature upon catalase activity depended upon the duration of the storage period, which varied with the temperature." Storage at 0° C. for 6 months resulted in greater activity in four varieties of pears. Reed (*15*) reported no catalase in green pineapples, some in hard yellow ones, and more in fully ripe ones.

In studies of the effect of maturity and storage on the oxidase and catalase activity of apples, Hinton (*10*) found that oxidase activity decreased during ripening on the tree, and markedly but slowly during storage. Catalase increased during storage, but in some cases decreased during the later stages. The rate of increase was highest during the early part of storage and fell more or less rapidly during the storage period. The later the date of picking the higher was the early rate of increase and the steeper the subsequent fall. With fruit picked at three stages of maturity the catalase activity was lowest in the middle picking. Hinton thought that this was probably due to lower temperatures prevailing prior to the second picking.

METHODS OF PROCEDURE

None of the fruit used in the present tests was canned. In order to make it possible to correlate oxidase and catalase activity with the previously observed behavior of the canned product, maturity and handling of the various lots of pears were similar to those reported in a previous paper (*3*). The oxidase activity was determined iodometrically as described by Guthrie (*6*) and is reported as cubic centimeters of N/100 sodium thiosulphate per 10 cc of juice, except as noted in the text. Catalase activity was determined by a water-displacement method similar to that described by Heinicke (*9*) and is reported as cubic centimeters of oxygen liberated in 5 minutes by 1 cc of juice. All enzyme determinations were made on the unripened fruit. Preliminary determinations made on the unripened fruit in storage at

32° F. and at daily intervals during ripening at 65° showed that the enzyme activity varied with the degree of ripeness. At the optimum stage for canning, oxidase activity was slightly greater than during storage at 32°, while catalase activity, which often increased at first, had fallen below that of the unripened fruit. In order to insure uniformity of sampling, unripened fruit was used. The methods of procedure have been described in detail by the authors in another paper (*4*).

EXPERIMENTAL DATA

EFFECT OF MATURITY ON ENZYME ACTIVITY

Bartlett pears were harvested at three stages of maturity, from three localities, and stored immediately at 32° F. Samples from each

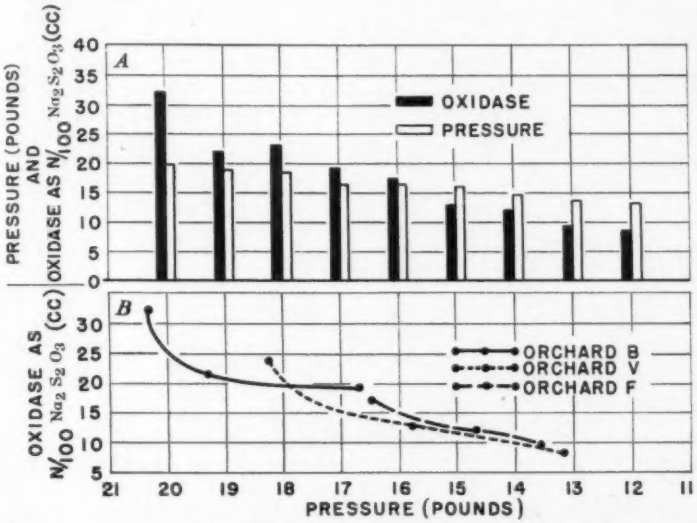

FIGURE 1.—Correlation between oxidase activity and maturity of nine lots of recently harvested Bartlett pears: *A*, Oxidase activity and pressure test of fruit from the three orchards arranged according to decreasing pressure; *B*, oxidase activity plotted against pressure test of fruit from individual orchards.

of the three localities were taken on the same dates, but owing to differences in elevation and soil conditions, the stages of maturity were not comparable. Oxidase and catalase determinations were made at harvest and at intervals during storage.

A very strong positive correlation was found between maturity, as measured by the pressure test, and oxidase activity, as is shown in figure 1. The pressure readings of the fruit during harvest ranged from 20.3 to 13.2 pounds, a drop of 35 percent, while the oxidase activity ranged from 32.8 to 7.9 cc of sodium thiosulphate, a drop of 76 percent.

No correlation was evident between degree of maturity, as measured by the pressure test, and catalase activity when the nine lots were arranged according to decreasing pressure. However, as shown in figure 2, when the lots were arranged by orchards the catalase activity

was low at the second picking and higher at the first and third pickings for two orchards. In the third orchard, in which the pressure reading was down to 16.5 pounds at the first picking, the catalase was low and continued to increase in the later pickings, which is in agreement with the hypothesis that catalase probably decreases as maturity advances, up to a definite stage, and then increases.

ORCHARD LOCATION AND ENZYME ACTIVITY

The orchards from which the pears used in the maturity studies were taken represent different growing conditions. Orchard F is a sandy loam, and fruit grown on this type of soil matures earlier than fruit grown on the type represented by orchard V, a medium-heavy loam more nearly typical of Wenatchee Valley orchards. Orchard B is also a medium-heavy loam but is situated at an elevation approximating 2,400 feet, whereas the elevation of the other two orchards is only 850 feet.

While the pears from the three orchards were not exactly comparable in maturity, there appear to be greater differences in enzyme activity than would be expected from maturity differences alone. For each pound drop in pressure there was an average drop in oxidase activity of 2.7 cc, 3.0 cc, and 3.8 cc in orchards F, V, and B, respectively, the greatest drop being in the least mature fruit. If the third picking of orchard B is compared with the first picking of orchard F and with the second picking of orchard V, at 16.7, 16.5, and 15.8 pounds, respectively—the three pickings most nearly comparable in maturity as measured by the pressure test—a difference will be noted of 0.85 cc of sodium thiosulphate in oxidase activity for each 0.1 pound difference in pressure between orchards B and F, 0.64 cc between orchards F and V, and 0.69 cc between orchards B and V. These values appear to be significantly higher than a corresponding difference in pressure would indicate in the individual orchards, where a maximum difference of 0.38 cc is noted for a similar drop in pressure. It thus appears that orchard differences would probably militate against setting an arbitrary oxidase figure for maturity (fig. 1, *B*). Seasonal conditions may also be a factor.

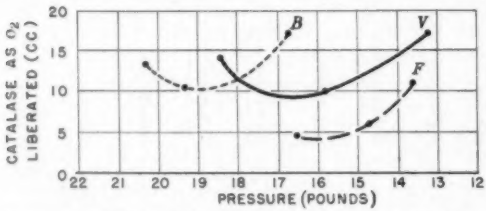

FIGURE 2.—Effect of maturity on catalase activity of Bartlett pears from different orchards.

EFFECT OF STORAGE ON ENZYME ACTIVITY

Bartlett pears from three orchards, harvested at three stages of maturity from each, were stored at 32° F. immediately after harvest. In table 1 are given the results of oxidase and catalase determinations at harvest and at intervals during storage. One lot, the first picking of orchard V, fluctuated irregularly throughout the storage period. This irregularity may have been due to spray materials on the fruit at harvest. With this exception, the oxidase and catalase activity were usually greater after storage than at harvest.

The effect of immaturity of fruit at harvest on its oxidase activity was magnified by holding the fruit in cold storage. This is shown graphically in figure 3, in which B (above) and V (below) represent fruit grown at elevations of about 2,400 and 850 feet, respectively. Fruit from orchard B was less mature than that picked on the same date from orchard V, and the oxidase increased more in storage. Also, the less mature fruit from orchard B showed a greater increase than the more mature fruit from the same orchard. It is significant that fruit harvested with a pressure test of 17 to 15 pounds (the pressure previously recommended for harvesting pears for canning), or below, showed comparatively little increase in oxidase activity during storage at 32° F. The greater oxidase activity in the early-picked fruit, especially after cold storage, accounts for the darker color observed in immature fruit when canned, and gives a basis for recommendations against holding immature Bartlett pears for long periods before canning. Catalase activity during storage was less closely associated with maturity at harvest than was oxidase activity.

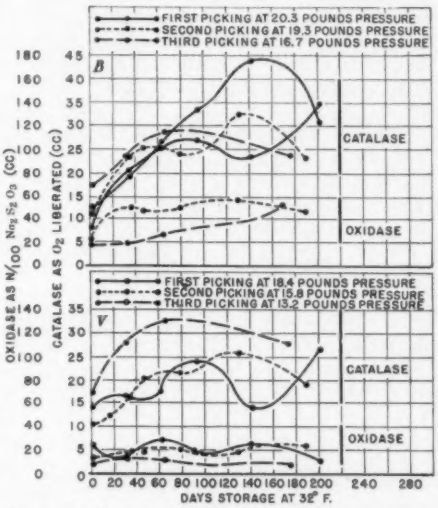
FIRST PICKING AT 20.3 POUNDS PRESSURE
SECOND PICKING AT 19.3 POUNDS PRESSURE
THIRD PICKING AT 16.7 POUNDS PRESSURE
B
CATALASE
OXIDASE
FIRST PICKING AT 18.4 POUNDS PRESSURE
SECOND PICKING AT 15.8 POUNDS PRESSURE
THIRD PICKING AT 13.2 POUNDS PRESSURE
V
CATALASE
OXIDASE
OXIDASE AS N/100 $Na_2S_2O_3$ (CC)
CATALASE AS O_2 LIBERATED (CC)
0 20 40 60 80 100 120 140 160 180 200 240 280
DAYS STORAGE AT 32° F.

FIGURE 3.—Effect of maturity on oxidase and catalase activity of Bartlett pears at harvest and during storage. Orchard B at an elevation of about 2,400 feet; orchard V at an elevation of about 850 feet.

TABLE 1.—*Oxidase and catalase activity of Bartlett pears as influenced by maturity and storage*

Orchard	Pressure test [1]	Oxidase activity [2] after indicated number of days at 32° F.						Catalase activity [3] after indicated number of days at 32° F.					
		0	32	57	86	135	189	0	32	57	86	135	189
	Pounds	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
B	20.3	32.8	83.5	103.0	109.0	95.0	141.0	13.5	19.5	26.8	33.6	44.4	31.1
	19.3	21.8	50.5	47.0	50.0	57.0	45.5	10.6	22.0	25.6	24.4	32.6	23.9
	16.7	19.1	18.5	26.5	------	------	52.0	17.4	23.6	28.7	------	------	24.2
V	18.4	23.7	14.2	28.0	16.5	24.5	10.0	14.3	16.8	17.3	24.2	13.9	27.1
	15.8	12.9	19.5	18.0	20.0	18.5	23.5	10.3	16.3	20.2	21.6	26.0	19.4
	13.2	7.9	13.0	11.5	------	------	7.0	17.1	27.6	33.0	------	------	28.0
F	16.5	17.4	29.0	33.5	31.5	46.0	57.0	4.7	9.1	12.0	15.2	23.3	13.3
	14.7	11.8	17.5	15.0	18.0	25.0	20.0	6.3	10.0	11.0	14.5	16.8	9.1
	13.6	9.5	25.5	14.5	------	------	19.0	11.0	14.2	19.6	------	------	16.0

[1] Pounds pressure at harvest.
[2] N/100 sodium thiosulphate per 10 cc of juice.
[3] Oxygen liberated in 5 minutes by 1 cc of juice.

ENZYME ACTIVITY DURING THE GROWING SEASON

In 1936 the maturity studies were expanded to include a wider range of maturity and were limited to one orchard. Pear fruits were

harvested from the tree in orchard V that was used in 1935. Fruit for enzyme studies was picked June 11 and at intervals thereafter until September 9, when the pressure test was 11.6 pounds. During this time the average weight per fruit increased from 19.1 g to 270.5 g. The oxidase activity of the very immature fruit was so great that it became necessary to reduce the amount of juice to 1 cc for each determination. Otherwise the procedure was the same as in the preceding year. Samples for enzyme determinations were taken immediately after the fruit was harvested.

TABLE 2.—*Oxidase and catalase activity and rate of growth of Bartlett pears during the growing season in 1936*

Date sampled	Weight per fruit	Pressure test	Oxidase (N/100 sodium thiosulphate per 1 cc of juice)	Catalase (O_2 liberated in 5 minutes per 1 cc of juice)	Date sampled	Weight per fruit	Pressure test	Oxidase (N/100 sodium thiosulphate per 1 cc of juice)	Catalase (O_2 liberated in 5 minutes per 1 cc of juice)
	Grams	*Pounds*	*Cc*	*Cc*		*Grams*	*Pounds*	*Cc*	*Cc*
June 11	19.1		24.0	24.0	Aug. 14	173.8	17.9	3.2	9.0
June 24	33.7		13.6	17.4	Aug. 27	230.6	15.4	2.5	12.0
July 21	93.5		6.2	9.8	Sept. 9	270.5	11.6	1.5	21.0
Aug. 4	151.0		4.2	9.9					

The effect of maturity on enzyme activity is shown in table 2 and figure 4. Oxidase activity declined very rapidly during the early part of the season and decreased more slowly as the fruit approached maturity. Catalase activity decreased until a pressure test of 17.9 pounds was reached, and then increased until the activity at the close of the experiment approximated that of the first sampling.

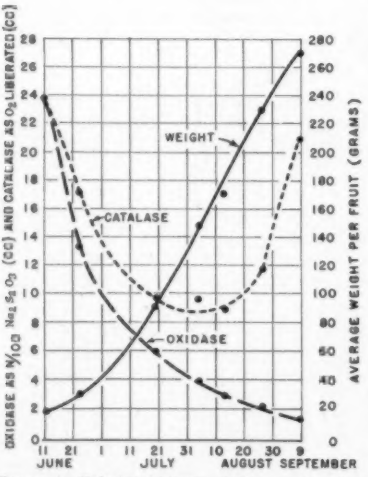

FIGURE 4.—Relation between catalase and oxidase activity and size of Bartlett pears during the growing season.

In the discussion of orchard location and enzyme activity it was pointed out that orchard differences would probably militate against setting an arbitrary oxidase figure as an index of maturity. In this connection it is of interest to compare the oxidase activity of the fruit from orchard V for the 2 years. Fruit taken from the same tree during 1935 and 1936, at pressure tests of 15.8 and 15.4 pounds, gave oxidase readings of 12.9 cc and 11.0 cc, respectively. The fruit was picked on August 26 in 1935 and on August 27 in 1936. The oxidase readings and the pressure tests indicate that the two seasons were fairly comparable. However, if the length of time from full bloom to harvest is taken as the index of maturity, the 1936 fruit, which was picked 122 days after full bloom, should have remained on the tree

6 days longer in order to reach the 128 days allowed the fruit in 1935. The oxidase activity would then have been less, and the difference in activity between the 2 years greater. While the difference in oxidase activity between the 2 years as given above may not be particularly significant, it appears unlikely that seasonal, orchard, and growing conditions will permit arbitrary oxidase limits to be set that will be applicable as a general index of the maturity at which Bartlett pears should be harvested.

The catalase activity on August 26, 1935, and August 27, 1936, was 10.3 cc and 12.0 cc, respectively. The former was the lowest reading recorded for the year 1935. Since samples were taken 12 days before and 17 days after the August 26 sampling, it may not be the absolute minimum for the year. In 1936 a minimum of 9.0 cc was recorded on August 14, and at time of sampling, on the 27th, the activity was increasing.

DISCUSSION

The decrease in oxidase activity in Bartlett pears as the fruit approached maturity is in agreement with the observed behavior of pears used for canning. Previously it was reported (*3*) that immature pears when canned gave a product grading toward a pale orange yellow in contrast to the clear cream yellow of more mature fruit. When the immature fruit was held in cold storage for long periods before ripening, the undesirable color increased. Practices that gave a dark, undesirable color in the canned product also gave increased oxidase activity, and practices that reduced darkening reduced oxidase activity. Where initial activity was low, it remained so during storage; but if the activity was high at the beginning of storage, it increased later so that differences in maturity were exaggerated by cold storage. The decrease in oxidase activity as the fruit approached maturity is in accord with the finding of Ajon (*1*) with citrus fruits, of De Villiers (*2*) with grapes, and of Hinton (*10*) with apples.

Catalase activity also was high in immature pears and decreased as the fruit approached harvest maturity. However, if the fruit was permitted to remain on the tree past the commercial harvest season catalase activity increased until the fruit was tree ripe, at which time the activity approximated that observed earlier in the season. Fruit picked at intervals representing early, medium, and late commercial maturity gave lower catalase activity at the second picking. These results were duplicated in different orchards and in different years, definitely indicating that the normal catalase curve of growing pear fruits is a U-shaped curve and that if the period of sampling were lengthened the extremities of the curve would presumably be extended.

The point of minimum activity of catalase varied in intensity and in relation to pressure test. This may be seen in figure 2, in which is shown the minimum for different orchards. Orchards B and V approached nearly the same minimum but reached it at different pressure tests. In orchard F the minimum was much lower. The minimum activity recorded for orchard V for the 2 years was 10.3 cc and 9.0 cc, respectively. If samples had been taken at shorter intervals a closer agreement between the minimum activity for the 2 years might possibly have been recorded.

While catalase activity does not appear to be correlated with color in the canned product it may be indirectly related to quality, since

the low point in the catalase curve occurs near the stage at which the fruit should be picked for canning in order to secure best results.

The low point in the catalase curve may also indicate a critical stage in the metabolism of the fruit, since it coincides with the period of minimum respiratory intensity (*4*) and also with the pressure test usually recommended in the Wenatchee district for the harvesting of Bartlett pears for best dessert and storage quality.

These results with pears lead the writers to suspect that the catalase curve of apples may also be a U-shaped curve and that the low results reported by Hinton (*10*) in his middle picking of apples may have been due to this characteristic of the catalase activity rather than to the cold weather to which he ascribed his results.

That the low points in the catalase curves in the present work are not due to low temperatures just before harvest may be seen from an examination of figure 2, which shows that fruit from different orchards, exposed to similar temperatures, reached the minimum in catalase activity at different times. Furthermore, in figure 4 the mean temperatures for the 7 weeks preceding harvest were 62°, 69°, 75°, 76°, 76°, 66°, and 70° F., respectively. Any correlation that might be found in this case between temperature and catalase activity of the fruit while still on the tree would evidently be a negative one.

It is not particularly surprising that the catalase curve should be U-shaped. Overholser (*14*) reported that immature Bartlett pears were high in catalase, which decreased as maturity advanced. His results indicated a continuous decrease. However, a study of his data permits some interesting comparisons to be made. Since it is not known how mature his fruits were at the time of final sampling, it may be possible that he discontinued sampling before the rise in catalase began. This hypothesis is supported by the fact that on May 28 the average weight of his pears was 15.2 g; on June 11 the average weight of the authors' pears was 19.1 g. Assuming that the rate of growth before the first sampling was approximately equal in the two cases, then the season of his study was about 2 weeks earlier than the season in which this study was made. If this is true, he discontinued sampling approximately 110 days after full bloom. The minimum activity recorded in the present study was 109 days after full bloom. If the number of days from full bloom be taken as the measure of maturity, he discontinued sampling at the stage of maturity at which the least activity was found in the work covered by this paper, and his results might be said to agree with those presented here. His report of 8.6 cc and 8.4 cc of oxygen liberated on July 27 and August 4, respectively, would indicate the low points in the catalase curve had been reached. Overholser mentioned that his results did not agree with Reed's findings of increase of catalase activity with ripening in the case of pineapples, when green, hard yellow, and fully ripe fruits were used.

When Bartlett pears are permitted to remain on the tree until tree-ripe, there is a tendency for the flesh to break down at the core. Harding (*8*) and Neller (*12*) have reported increased catalase activity of apples in storage as they approach the break-down stage. It is possible that the accumulation of acetaldehyde and alcohol which takes place as fruit approaches the break-down stage stimulates catalase production, causing the rise in activity. This viewpoint is strengthened by the increase in catalase activity of fruit subjected to alcohol vapors (*4*).

SUMMARY

The effect of maturity and handling practices on the oxidase and catalase activity of Bartlett pears has been studied in relation to the observed behavior of pears used for canning.

Oxidase activity decreased throughout the growing season.

The catalase activity of Bartlett pears from the time they were very small until they were tree-ripe formed a U-shaped curve. The minimum activity occurred near the period at which the fruit should be harvested for canning.

High oxidase activity is correlated with practices that give an undesirable color in canned pears, and low oxidase activity is correlated with practices that give a desirable color. It therefore appears that high oxidase activity may be the principal cause of poor color in the canned product.

Catalase activity apparently is unrelated to color development in canned Bartlett pears but may be related indirectly to quality since best results are obtained with fruit picked when the low point in the catalase curve is reached or soon thereafter.

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A COMPARATIVE STUDY OF AN EARLY, A MEDIUM, AND A LATE STRAIN OF TIMOTHY HARVESTED AT VARIOUS STAGES OF DEVELOPMENT [1]

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INTRODUCTION

In Ohio, timothy and timothy mixtures make up the largest percentages of the hay crop. In 1934, 25.5 percent of the hay acreage harvested was timothy, and 37.4 percent was timothy-clover mixture.[2]

At the Missouri Agricultural Experiment Station it was found that the palatability of timothy fed to steers was greater when the crop was cut early than when it was cut nearly mature (*10*),[3] the order of preference shown by the steers for the hay being the same as the order in which the timothy was cut.

At the Ohio Agricultural Experiment Station (*7*) it was found that the amounts of both vitamin B and vitamin G are positively correlated with leafiness, greenness, and the protein content of alfalfa, clover, and timothy plants, and that these vitamins decrease as the season advances.

In an experiment conducted with sheep at the Ohio station (*2, p. 179*) it was found that—

> the time at which timothy was cut for hay had a definite influence on its value for sheep feeding. Timothy cut when not more than one-third of the heads were in blossom was fully one-third better than timothy cut after the blossoms had fallen and as the seed was ripening. From a practical standpoint, the early cut timothy was a usable roughage whereas the late cut * * * was decidedly inferior.

With few exceptions, time-of-harvesting and feeding experiments have been carried on with ordinary unimproved timothy (*Phleum pratense* L.). The development of improved varieties or strains at the Timothy Breeding Station at North Ridgeville, Ohio (*3*),[4] and at other agricultural experiment stations in the United States and elsewhere, and their probable distribution to farmers, made it desirable to compare certain ones, especially early and late strains, with ordinary timothy in a time-of-harvesting experiment. The results of such an experiment are reported in this paper.

MATERIALS AND METHODS

STRAINS USED

The timothy strains used in this experiment were F. C. 11901,[5] a medium early maturing selection; F. C. 12368, a late-maturing selec-

[1] Received for publication June 1, 1937; issued April 1938. Contribution from the Division of Forage Crops and Diseases, U. S. Department of Agriculture, cooperating with the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] The authors are indebted to Dr. J. I. Falconer, chairman of the Department of Rural Economics, Ohio State University, for these data.

[3] Reference is made by number (italic) to Literature Cited, p. 363.

[4] The Division of Forage Crops and Diseases; Bureau of Plant Industry, U. S. Department of Agriculture, and the Ohio Agricultural Experiment Station cooperating.

[5] Accession number of the Division of Forage Crops and Diseases. If introduced into production, F. C. 11901 will be known as Marietta timothy.

tion; and the ordinary unimproved timothy grown in this country and designated herein as medium timothy. The selections were developed by the senior author at the Timothy Breeding Station where the time-of-harvesting experiment was made.

At North Ridgeville in northern Ohio, medium timothy is usually in full bloom from about June 22 to July 1. The early strain is about 4 days earlier and the late strain about 7 days later than medium timothy.

PLOT TECHNIQUE

The land was plowed in the summer of 1930, and late in August an application of 180 pounds per acre each of nitrate of soda and 16-percent superphosphate was applied to the seedbed. On September 18, plots of the three strains of timothy were sown at the rate of 5 pounds of seed per acre with a nurse crop of rye. Medium red clover, at the rate of 10 pounds per acre, was sown on all plots the following spring. The rye was harvested for grain in 1931. The individual plots from which hay was harvested were 49.5 by 5 feet. There were 24 plots of each of the three strains of timothy, quadruplicate plots being harvested at each of six harvest periods.

The six harvests were made during a period of 35 to 38 days, the first one being made when medium timothy was partly headed and the last one when its seed was mature. The six periods have been designated by the letters A to F, respectively. The actual dates of harvest, together with the condition of the timothy, are shown in table 1. All harvests were made according to this schedule except the aftermath of 1932, which was harvested on August 31.

TABLE 1.—*Date of harvesting and condition of each strain, 1932–34*

[Time of harvest was determined by the condition of ordinary timothy]

Year	Period of harvest	Date of harvest	Condition of indicated strain of timothy		
			F. C. 11901 (early)	Ordinary (medium)	F. C. 12368 (late)
1932 [1]	A	June 14	Headed	Partly headed	No heads.
	B	June 18	Full bloom	Fully headed	Few heads.
	C	June 22	....do	Early bloom	Nearly headed.
	D	July 1	Past bloom	Just past full bloom	Full bloom.
	E	July 12	60 percent heads becoming straw color.	Seed beginning to mature.[2]	Just past full bloom.
	F [3]	July 20	Mature	Seed mature [4]	50 percent heads becoming straw color.
1933	A	June 9	Nearly headed	Partly headed	No heads.
	B	June 14	Early bloom	Fully headed	Few heads.
	C	June 20	Full bloom	Early bloom	20 percent heads emerged.
	D	June 29	Past bloom	Just past full bloom	Early bloom.
	E	July 8	85 percent heads becoming straw color.	Seed beginning to mature.[2]	Past bloom.
	F	July 17	Mature	Seed mature [4]	30 percent heads straw color.
1934	A	June 11	Very early bloom	Partly headed	No heads.
	B	June 14	Early bloom	Fully headed	Few heads.
	C	June 19	Full bloom	Early bloom	Nearly headed.
	D	June 28	Past bloom	Just past full bloom	Early bloom.
	E	July 9	90 percent heads becoming straw color.	Seed beginning to mature.[2]	Past full bloom.
	F	July 16	Mature	Seed mature [4]	25 percent heads straw color.

[1] The crop of aftermath obtained in 1932 was harvested on Aug. 31.
[2] About 10 percent of the earliest heads beginning to turn straw color.
[3] At the last date of harvesting the main crop of mixed hay in 1932, most of the clover heads and a large proportion of the lower leaves on the clover stems were dry and brown.
[4] Nearly all heads straw color.

The percentages of timothy, clover, and weeds in the dry hay were determined for the 1932 and 1933 crops by sorting representative square-yard samples from each plot; the 1934 crop of clear timothy required no sorting.

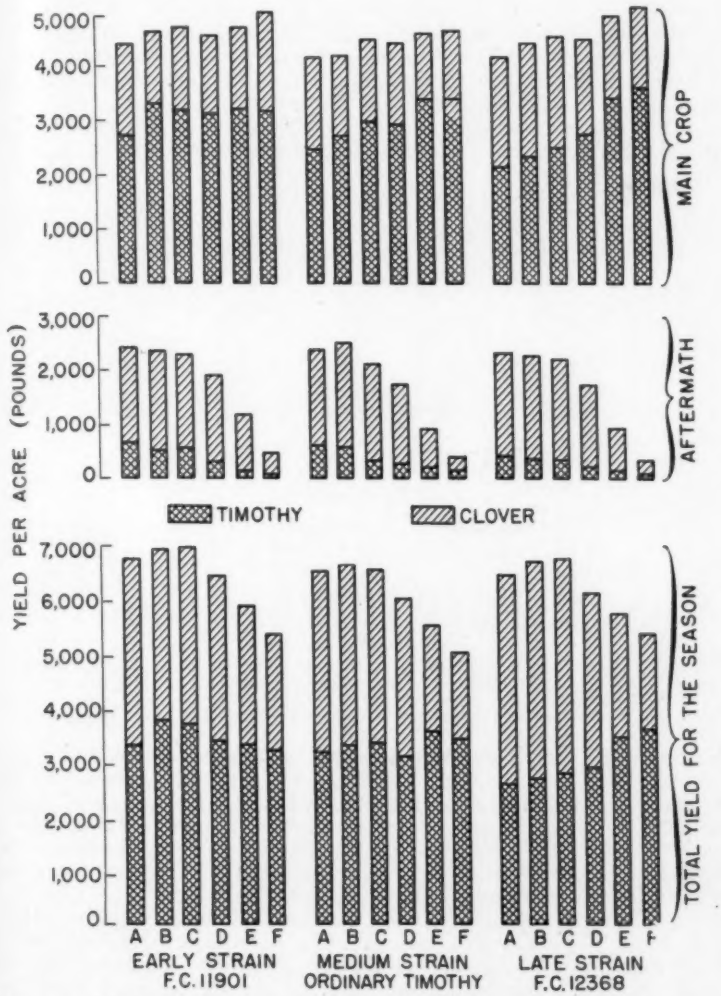

FIGURE 1.—Yields of timothy and of clover, in the main crop and the aftermath, and the total in both crops harvested in 1932. Letters at bottom indicate harvesting periods shown in table 1.

The protein (nitrogen×6.25) content of the hay as harvested and as sorted was determined by the Department of Agronomy of the Ohio Agricultural Experiment Station.

On May 10, 1934 (the third harvest season), 150 pounds per acre each of sulphate of ammonia and 20-percent superphosphate were applied as a top dressing on the timothy.

INTERPRETING THE DATA

The data obtained consisted of the yields of hay, the proportions of timothy and clover in the mixed hay, the percentages and yields of protein, and the color analyses and grades of hay. In interpreting these data, the following facts should be borne in mind:

(1) Because of the differences in their response to days of various lengths (*4*) early, medium, and late timothy growing in different

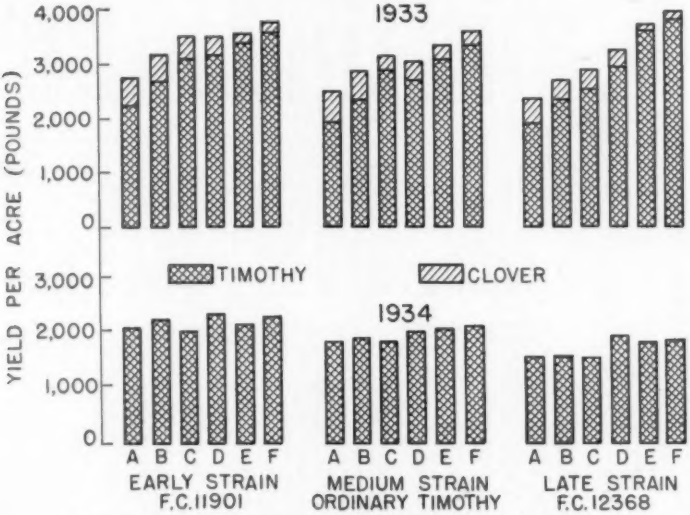

FIGURE 2.—Yields of timothy and clover hay in 1933, and of timothy in 1934.

latitudes (*5*) vary in relative dates of heading and of blooming and period of development, and caution should be used in predicting the probable results to be expected at locations north or south of North Ridgeville.[6] Experiments so far conducted indicate that in general early varieties of timothy are well adapted to both southern and northern latitudes within the range where timothy is grown, whereas late varieties are adapted to northern latitudes only.

(2) The three strains of timothy were grown in mixture with medium red clover (*Trifolium pratense* L.). There is no assurance that yields comparable to those obtained could have been expected had the clover been omitted. Nilsson-Leissner (*9*), in Sweden, found that the growth response of any variety of timothy grown in mixture with clover may be entirely different from that of the same variety grown alone. However, the yields of the timothy fractions of the hay may give some indication, at least, of what may be expected in the absence of a companion crop of clover.

[6] North Ridgeville is located at lat. 41°23′ N.

(3) The amount and distribution of the rainfall during the spring and early summer have an influence upon the yields of timothy. The yield in 1934 was undoubtedly reduced by the abnormally dry weather during May and the first half of June. Table 2 shows the records of precipitation at the Cleveland, Ohio, station of the Weather Bureau, United States Department of Agriculture, which is located about 20 miles east of the Timothy Breeding Station at North Ridgeville and at approximately the same elevation.

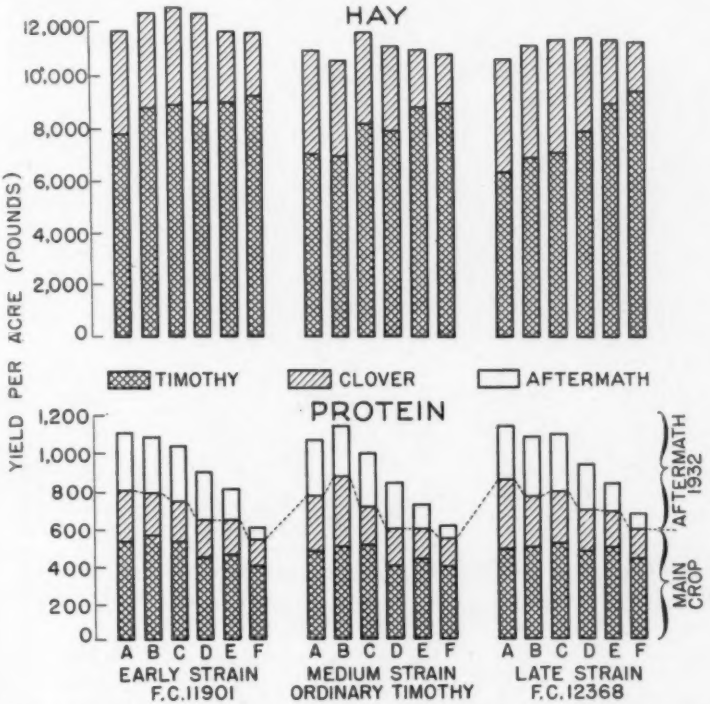

FIGURE 3.—Total yields of hay and yields of protein produced by the timothy and by the clover in 3 years.

TABLE 2.—*Monthly and annual precipitation at the United States Weather Bureau station at Cleveland, Ohio*

Year	Monthly precipitation												Annual precipitation
	January	February	March	April	May	June	July	August	September	October	November	December	
	In.	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
1932	3.46	1.10	3.39	2.09	3.71	1.88	2.42	4.31	1.45	3.06	2.63	3.77	33.27
1933	1.40	1.99	3.51	2.42	2.86	.39	1.26	1.98	2.73	.97	3.06	1.60	24.17
1934	1.88	1.02	2.43	2.43	.58	[1] 2.25	2.70	2.46	2.12	.99	1.40	1.84	21.81
Mean, 1871 to 1935	2.54	2.38	2.75	2.47	3.00	3.16	3.45	2.90	3.21	2.70	2.57	2.40	33.53

[1] In June 1934, 97.4 percent of the rainfall occurred on and after June 18.

TABLE 3.—*Yields per acre of hay, 1932–34*

Year	Crop	Yield per acre at indicated harvest period (main crop)																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
1932	Main crop:	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
	Timothy fraction [1]	2,735	3,317	3,188	3,143	3,227	3,196	2,565	2,812	3,025	2,852	3,419	3,407	2,155	2,367	2,608	2,778	3,365	3,582
	Clover fraction	1,703	1,336	1,543	1,472	1,528	1,830	1,601	1,431	1,490	1,492	1,235	1,315	2,034	2,099	2,091	1,779	1,552	1,529
	Total (mixed hay)	4,438	4,653	4,731	4,615	4,755	5,026	4,166	4,243	4,515	4,344	4,654	4,722	4,189	4,466	4,699	4,557	4,917	5,111
	Aftermath: [2]																		
	Timothy fraction [1]	645	465	542	281	138	62	629	545	383	299	203	76	435	361	335	190	150	79
	Clover fraction	1,771	1,855	1,737	1,611	1,049	360	1,766	1,950	1,747	1,438	738	294	1,885	1,953	1,875	1,519	768	278
	Total (mixed hay)	2,416	2,320	2,299	1,892	1,187	422	2,395	2,495	2,130	1,737	941	370	2,320	2,314	2,210	1,709	918	357
1933	Main crop (only):																		
	Timothy fraction [1]	2,225	2,692	3,086	3,154	3,374	3,584	1,916	2,343	2,856	2,671	3,065	3,350	1,886	2,351	2,541	2,932	3,599	3,791
	Clover fraction	488	462	378	303	187	193	568	522	266	368	239	223	463	332	321	277	141	162
	Total (mixed hay)	2,713	3,154	3,464	3,457	3,561	3,777	2,484	2,865	3,122	3,039	3,304	3,573	2,349	2,683	2,862	3,209	3,740	3,953
1934	Main crop (only):																		
	Timothy	2,095	2,248	2,046	2,343	2,152	2,284	1,830	1,922	1,841	2,001	2,049	2,070	1,674	1,608	1,659	1,915	1,798	1,857
1933–34	Total	11,662	12,375	12,540	12,307	11,655	11,509	10,875	11,525	11,608	11,121	10,948	10,735	10,532	11,131	11,430	11,390	11,373	11,278
	Total minus aftermath [3] in 1932	9,246	10,055	10,241	10,415	10,468	11,087	8,480	9,030	9,478	9,384	10,007	10,365	8,212	8,817	9,220	9,681	10,455	10,921

[1] Includes the small percentage of weeds.
[2] Harvested all on one date, Aug. 31.
[3] It is assumed that if the aftermath in 1932 had not been harvested there would have been no change in the yields in 1933 and 1934.

YIELDS OF HAY

The yields of hay are recorded in table 3 and illustrated graphically in figures 1, 2, and 3. The first season's crop of 1932 consisted of a main crop, of mixed timothy and clover, and an aftermath, mostly clover. The following year, 1933, the main crop of mixed hay contained only a small amount of clover, and the third year's crop was clear timothy.

In the main crop of mixed hay, except the plots of early timothy in 1932, the yields of the clover fraction were less for the last two harvest periods than for the earlier ones. This loss may have been due to the

FIGURE 4.—Plots of timothy and clover mixture. Right, early timothy (F. C. 11901) in early bloom. Left late timothy (F. C. 12368) only partly headed. Photographed June 16, 1932.

shattering of the leaves and other parts of the clover plants as they approached maturity (*11*).

The time of harvesting the main crop had a very marked effect upon the yields of the aftermath harvested August 31, the yields falling off most rapidly when the main crop was harvested after the C period (June 22). If the aftermath is included in the total yields of hay for the season, the advantage of early harvesting is evident, as shown in figure 1.

In 1933 (fig. 2), as in the main crop of 1932, the yields increased as harvesting took place later. Since in 1933 the crop consisted largely of timothy, late harvesting gave larger increases in yield than in 1932.

In 1934 (fig. 2) the effect of time of harvest on yield was less marked than in 1932 and 1933. The dry weather during May and June limited the growth in all plots. The early timothy suffered less from the drought than did the late timothy. Apparently the relatively early growth of the culms and the earlier heading and blooming of the early timothy enabled it to more nearly complete a normal growth before soil moisture became too much of a limiting factor. Had the

rainfall been normal it is probable the late timothy would have made higher yields at the D, E, and F periods of harvest. Later harvesting resulted in higher yields in all three timothy strains when the aftermath in 1932 was deducted from the total yields for 3 years (table 3).

The total yields of hay for the three seasons—aftermath included—are shown in figure 3. The early timothy produced more hay than either the medium or the late timothy, especially at the early periods of harvest. The total yields produced by the early strain for all 3

FIGURE 5.—Right, late timothy (F. C. 12368), maximum height 27 inches. Left, medium timothy (ordinary), maximum height 36 inches. Photographed June 21, 1933.

years, at comparable periods of harvest, were about 700 to nearly 1,200 pounds per acre greater than those produced by ordinary timothy.

Table 3 shows that in the first four periods of harvest of the main crop in 1932 there was an average of 487 pounds more clover per acre with the late than with the early timothy, and 497 pounds more with the late than with the ordinary timothy. This may be attributed to the relatively slow growth of the late timothy. Since clover adds to the feeding value of the mixed hay this may be a point in favor of late timothy (figs. 4 and 5).

PROPORTIONS OF DIFFERENT CONSTITUENTS IN THE HAY

Table 4 shows that in the first or main crop harvested in 1932, at all but the last two harvest periods, there was a larger percentage of clover in the late selection than in either the early selection or the medium timothy, which were not greatly different. Delaying the harvest of the ordinary and late timothy tended to decrease the percentage of clover in the first crop, but this is not apparent for the early timothy, possibly because of some error.

TABLE 4.—*Proportions of timothy, clover, and weeds in the two crops harvested in 1932 and in the crop harvested in 1933*

Year	Crop	Constituent at indicated harvest period [1] (main crop) of—																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1932	Main crop:																		
	Timothy	61.6	70.5	67.1	68.0	67.5	63.5	61.5	66.2	66.8	65.6	73.4	71.9	51.3	52.8	54.5	60.4	68.0	69.5
	Clover	38.4	28.7	32.6	31.9	32.1	36.4	38.4	33.7	33.0	34.3	26.5	27.8	48.6	47.0	44.5	39.0	31.6	29.9
	Weeds	.0	.8	.3	.1	.4	.1	.1	.1	.2	.1	.1	.3	.1	.2	1.0	.6	.4	.6
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Aftermath: [2]																		
	Timothy	26.3	19.4	23.6	14.4	11.3	14.7	26.1	21.7	17.5	17.1	21.3	20.5	18.6	15.3	14.4	11.0	15.7	22.1
	Clover	73.3	80.0	76.4	85.2	88.4	85.3	73.7	78.2	82.0	82.8	78.4	79.5	81.3	84.4	84.8	88.8	83.6	77.9
	Weeds	.4	.6	.0	.4	.3	.0	.2	.1	.5	.1	.3	.0	.1	.3	.8	.2	.7	.0
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1933	Main crop (only):																		
	Timothy	81.0	84.7	88.8	90.9	94.4	94.8	76.2	81.5	91.3	87.3	92.6	93.2	79.6	86.8	87.7	90.3	95.3	95.0
	Clover	18.0	14.7	10.9	8.8	5.3	5.1	22.9	18.2	8.5	12.1	7.2	6.2	19.7	12.4	11.2	8.6	3.8	4.1
	Weeds	1.0	.6	.3	.3	.3	.1	.9	.3	.2	.6	.2	.6	.7	.8	1.1	1.1	.9	.9
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] See table 1 for explanation of harvest periods.
[2] Harvested all on 1 date, Aug. 31.

In the second crop, or aftermath, harvested in 1932, and also in the crop of 1933, the differences in the percentage of timothy and clover for the different varieties show no consistent trends.

PROTEIN CONTENT OF THE HAY

The percentage of protein in the hay, and consequently its feeding value, was markedly influenced by the time of harvest.

The percentage of protein in the mixed hay, and also in the timothy and clover fractions, in the main crop of 1932 and in the crop of 1933, together with the percentages of protein in the timothy harvested in 1934, are presented in table 5 and are shown graphically in figures 6 to 10. The calculated yields of protein per acre are also presented in table 5 and are shown graphically in figures 3 and 11.

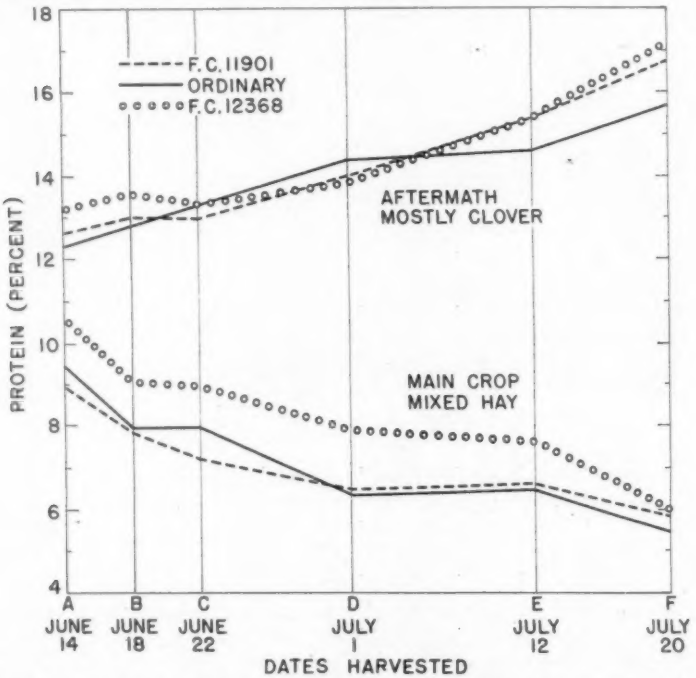

FIGURE 6.—Percentages of protein in the mixed hay produced by the main crop and by the aftermath in 1932.

PERCENTAGE OF PROTEIN

In both the timothy and the clover, the percentages of protein in all of the main crops were highest in the hay harvested at the earliest dates and gradually decreased, with minor exceptions in occasional samples, up to the time of latest harvest.

In each season, the percentage of protein in the mixed hay, in the timothy fraction of the mixed hay, and in the timothy alone, at any harvest period, differed but little for the early and medium timothy. This may be attributed to the tendency of the leaves on the stems of F. C. 11901 to remain almost as green as those of ordinary timothy (*6*) at comparable periods of harvest.

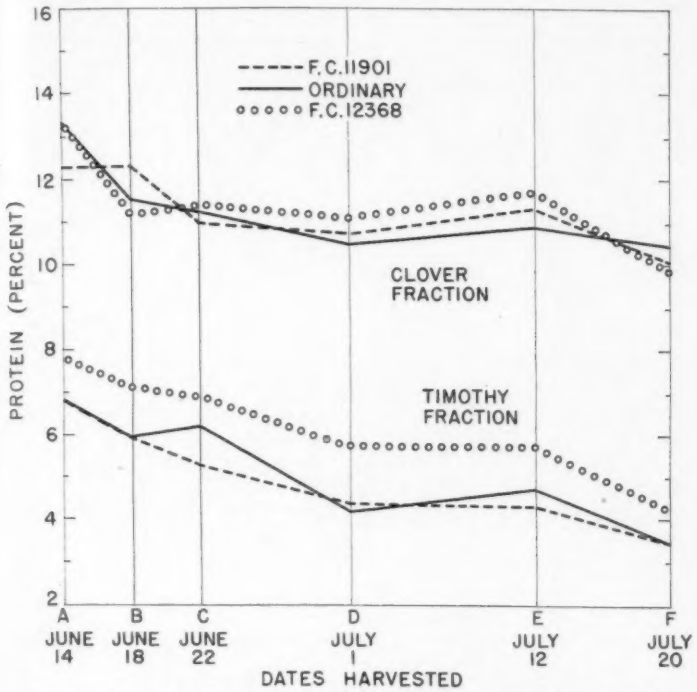

FIGURE 7.—Percentages of protein in the timothy and clover fractions of the main crop in 1932.

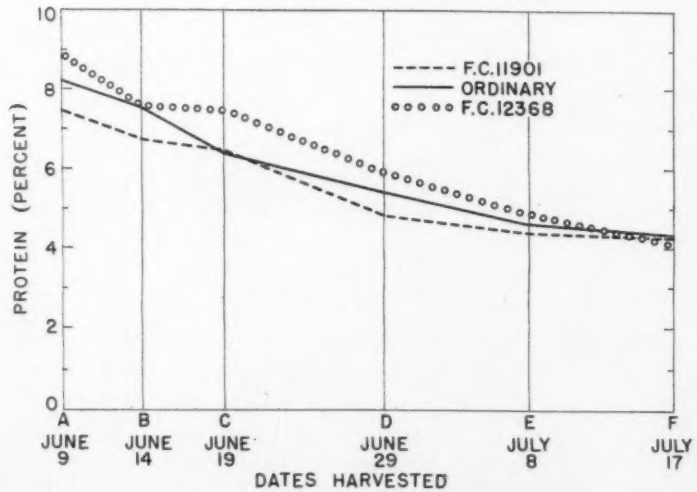

FIGURE 8.—Percentages of protein in the 1933 crop of mixed hay.

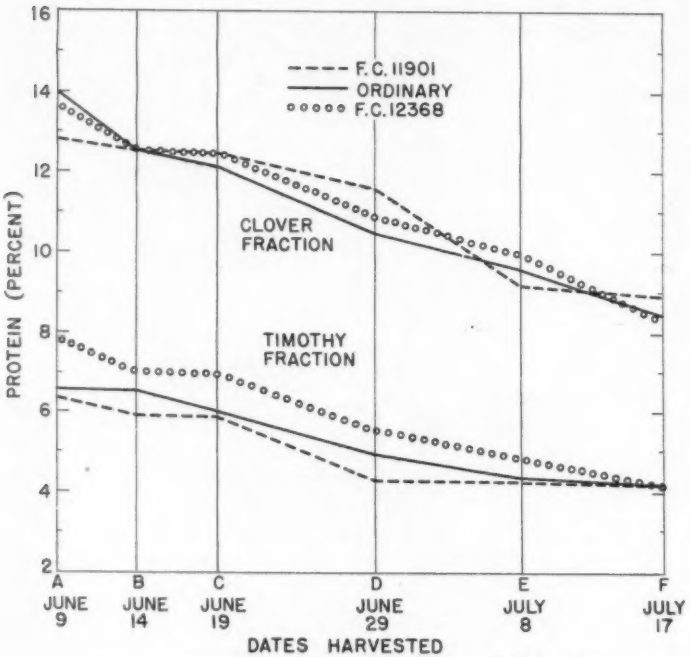

FIGURE 9.—Percentages of protein in the timothy and clover fractions in 1933.

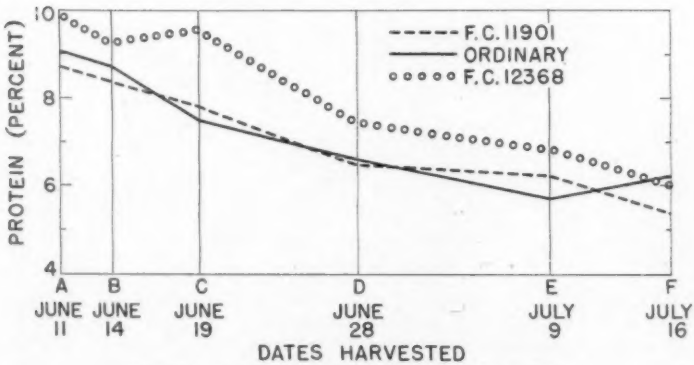

FIGURE 10.—Percentages of protein in the 1934 crop of timothy.

At all times, with minor exceptions, the late timothy, alone or in the mixed hay, contained a higher percentage of protein than the hay produced by the early or medium strains of timothy. The relatively high percentage of protein in the late timothy and clover mixture may be attributed in part to the larger proportion of clover which it contained, as well as to the fact that the percentage of protein was higher in its timothy fraction than in that of the early or medium timothy mixtures.

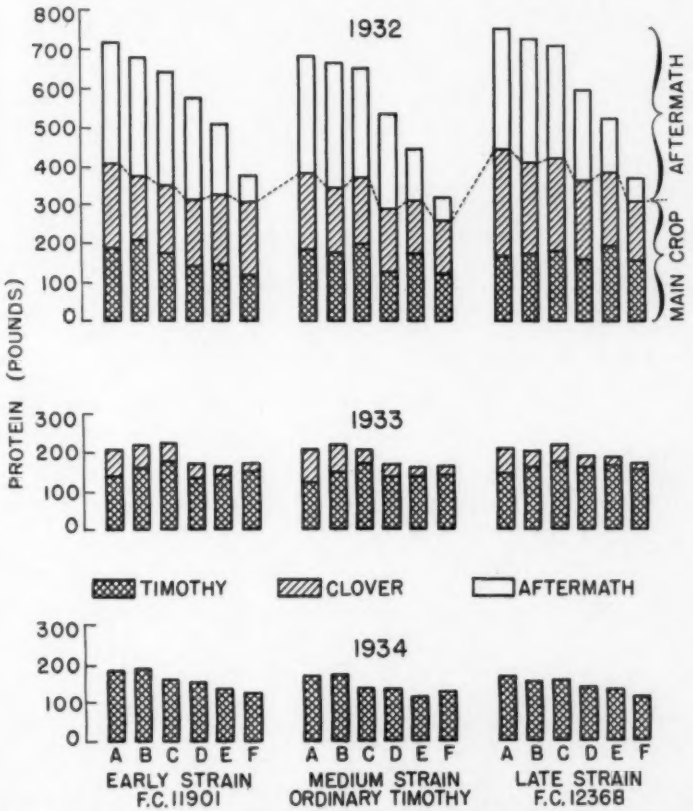

FIGURE 11.—Yields of protein per acre, produced by timothy and by clover, in 1932, 1933, and 1934.

The percentages of protein were higher in the timothy harvested in 1934 than in the timothy fractions in either 1932 or 1933, even though in both of these years the timothy was growing in mixture with clover. A grass grown in mixture with a legume usually contains a greater proportion of protein than one that is grown alone (*8*, *12*). The application of sulphate of ammonia made in the spring of 1934 may explain in part the relatively high percentages of protein in the timothy in that season (*13*). A second contributing factor was probably the

TABLE 5.—*Percentages and yields of protein per acre in the hay harvested in 1932–34*

Year	Crop	Protein per acre at indicated harvest period[1] (main crop) in—																	
		Early timothy (F. C. 11901)						Medium timothy (ordinary variety)						Late timothy (F. C. 12368)					
		A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
		Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1932	Main crop:																		
	Timothy fraction	6.81	6.02	5.40	4.49	4.48	3.57	6.92	6.13	6.30	4.32	4.88	3.57	7.89	7.27	7.04	5.84	5.84	4.37
	Clover fraction	12.41	12.41	11.05	10.81	11.39	10.19	13.45	11.56	11.39	10.58	10.99	10.53	13.45	11.21	11.50	11.21	11.78	9.90
	Total (mixed hay)	8.95	7.87	7.25	6.50	6.71	5.97	9.41	7.94	8.00	6.47	6.51	5.50	10.60	9.11	9.07	7.92	7.73	6.03
	Aftermath: Mixed hay[2]	12.68	13.02	13.02	14.00	15.47	16.86	12.38	12.84	13.37	14.47	14.65	15.70	13.25	13.66	13.37	13.89	15.40	17.21
1933	Main crop (only):																		
	Timothy fraction	6.35	5.88	5.88	4.31	4.31	4.25	6.52	6.52	6.06	4.95	4.43	4.25	7.81	6.99	6.99	5.54	4.84	4.25
	Clover fraction	12.87	12.47	12.47	11.59	9.21	8.97	14.10	12.47	12.17	10.43	9.61	8.51	13.69	12.47	12.47	10.84	9.96	8.39
	Total (mixed hay)	7.50	6.85	6.58	4.95	4.55	4.47	8.25	7.61	6.57	5.59	4.81	4.51	8.94	7.64	7.62	5.98	5.03	4.43
1934	Main crop (only): Timothy	8.79	8.39	7.81	6.56	6.21	5.42	9.18	8.79	7.53	6.61	5.76	6.21	9.93	9.23	9.58	7.42	6.84	5.99
		Lb.	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*	*Lb.*
1932	Main crop:[3]																		
	Timothy fraction[4]	186	200	172	141	145	114	177	172	191	123	167	122	170	172	177	162	197	157
	Clover fraction	211	166	171	159	174	186	215	165	170	158	136	138	274	235	240	199	183	151
	Total (mixed hay)	397	366	343	300	319	300	392	337	361	281	303	260	444	407	417	361	380	308
	Aftermath: Mixed hay[2]	306	302	289	265	184	71	297	320	285	251	138	58	307	316	296	237	141	61
1933	Main crop (only):																		
	Timothy fraction[4]	141	158	181	136	145	152	125	153	173	132	136	142	147	164	178	162	174	161
	Clover fraction	63	58	47	35	17	17	80	65	32	38	23	19	63	41	40	30	14	14
	Total (mixed hay)	204	216	228	171	162	169	205	218	205	170	159	161	210	205	218	192	188	175
1934	Main crop (only): Timothy	184	189	160	154	134	124	168	169	139	132	118	129	166	154	159	142	123	111
1932–34	Total	1,091	1,073	1,020	890	799	664	1,062	1,044	990	834	718	608	1,127	1,082	1,090	932	832	655

[1] See table 1 for explanation of harvest periods.
[2] Aftermath harvest all on 1 date, Aug. 31.
[3] Protein in the fractions of the aftermath was not determined.
[4] Includes the small percentage of weeds.

unusually dry season of 1934, preceded by a season with rainfall 25 percent below normal. In years of low rainfall there is likely to be an accumulation of nitrate nitrogen in the soil (*1*). An abundance of nitrate nitrogen in the soil would tend to increase the protein content of the timothy, especially if the growth subsequent to nitrogen assimilation is limited.

In the aftermath produced in 1932 and harvested on August 31, there was a gradual increase in the percentage of protein as the time of harvesting the main crop became later. This was to be expected, since the aftermath growth was relatively younger as the date of harvest for the main crop was advanced.

YIELD OF PROTEIN

The yields of protein attained a maximum at the A period of harvest in the first crop in 1932 and at the A, B, or C period in the crops of 1933 and 1934, but at the D, E, and F periods, even with greater yields of hay, the amount of protein became less (figs. 3 and 11).

In the aftermath of 1932 the percentage of protein increased as the time of harvesting the first crop was delayed, but since the yield of hay in the aftermath was smaller the later the first crop was harvested, the yield of protein in the aftermath was also smaller.

Since clover contains a higher percentage of protein than does timothy, and since the first crop and the aftermath of the first season contained much clover, the yields of protein per acre for the first season were determined largely by the clover fraction of the hay. In the second season's crop the clover fractions were relatively small and so had little influence on the protein content of the hay.

COLOR AND GRADE OF HAY

In 1934, samples of the hay collected at each time of harvest were analyzed for degree of green color and were graded according to the United States hay standards. These records (table 6) were made by the Division of Hay, Feed, and Seed, Bureau of Agricultural Economics, United States Department of Agriculture.

TABLE 6.—*Color analyses and grades of hay produced by early, medium, and late timothy at 6 stages of development in 1934*

Stage [1]	Date of harvest	Strain	Color reading or hue [2]	United States class and grade
A	June 11	F. C. 11901	8.81	U. S. No. 1 Extra Green Timothy.
		Ordinary	9.21	Do.
		F. C. 12368	9.27	Do.
B	June 14	F. C. 11901	8.75	Do.
		Ordinary	8.92	Do.
		F. C. 12368	9.25	Do.
C	June 19	F. C. 11901	8.84	Do.
		Ordinary	8.78	Do.
		F. C. 12368	9.23	Do.
D	June 28	F. C. 11901	6.94	Do.
		Ordinary	6.00	Do.
		F. C. 12368	7.78	Do.
E	July 9	F. C. 11901	4.74	U. S. No. 1 Timothy.
		Ordinary	4.21	U. S. No. 2 Timothy.
		F. C. 12368	5.90	U. S. No. 1 Timothy.
F	July 16	F. C. 11901	2.94	U. S. No. 3 Timothy.
		Ordinary	3.82	U. S. No. 2 Timothy.
		F. C. 12368	4.71	U. S. No. 1 Timothy.

[1] See table 1 for explanation of harvest periods.

[2] As the percentage of green color decreases, the color reading becomes lower. The complete reading for each sample is "8.81y," "9.21y," etc.

Each sample of hay was cured in a barn, where it was protected from sun, dew, and rain. If the hay had been cured in the field in the usual way, sometimes under relatively poor weather conditions, the ratings for color and grade, for some of the samples at least, probably would have been lower.

Grades of hay are based chiefly on the mixture of plants of which it is composed and on color. Since these samples were composed of clear timothy, the differences in the grades may be attributed entirely to the differences in the proportions of green parts.

As the season advanced and the proportions of dry brown leaves, stems, and heads increased, there was a gradual decrease in the color reading or hue of the hay. Because of the favorable conditions under which the samples were cured, all strains were green enough through June 28 to make hay classed as "U. S. No. 1 Extra Green Timothy." After that the grades were lower.

At all periods of harvest, the color reading, or hue, of the late strain was higher than that of either of the other two strains. At the A, B, and F periods of harvest, the medium strain was a little greener than the early strain. At the C, D, and E periods the early strain was slightly greener than the medium strain. This was due largely to the tendency of each leaf blade of the early strain to remain green somewhat longer than the corresponding leaf on a stem of the medium strain (*6*). The relatively low rating of the early strain at the last stage of harvest was in part due to the large number of mature or nearly mature heads.

SUMMARY AND CONCLUSIONS

An early selection of timothy (F. C. 11901), a late selection (F. C. 12368), and ordinary commercial or medium timothy were grown in mixture with medium red clover and harvested at six periods, representing different stages of maturity, for a 3-year period. A main crop and an aftermath of mixed timothy and clover were harvested in 1932; one crop, composed chiefly of timothy, in 1933; and a crop of clear timothy in 1934.

At North Ridgeville, Ohio, the early strain blooms and matures approximately 4 days earlier, and the late strain 7 days later, than ordinary timothy. Although the different harvest periods were not so spaced as to permit exact comparisons, in general the results indicate that the early timothy will produce yields of hay equal to or greater than the yields produced by the medium timothy when the latter arrives at the same stage of development several days later, and that the percentage and yield per acre of protein of the early timothy will equal or exceed that of the medium timothy. Likewise, the late selection harvested at any time will produce yields of hay approximately equal to the yields produced by the medium timothy at the same stage of development about a week earlier and containing a percentage and yield per acre of protein equal to that for the medium timothy.

If the main crops only are considered, the yields of hay increased as the timothy progressed from heading to maturity.

In 3 years, the early selection produced larger yields of hay than ordinary timothy at all periods of harvest. At the three earliest harvest periods the late selection produced less than the medium strain, but at the three latest periods it produced more.

When the aftermath is included in the total yields of hay the advantage of early harvesting, especially for the first season's crop, becomes apparent. Since the aftermath decreases rapidly after the C harvest period, the importance of efficient utilization of the aftermath through early harvesting of the first crop is indicated. The records of yields for the 3-year period—aftermath included—show more advantage in early harvesting for the early timothy than for the late timothy.

The percentage of protein in the hay declined in all three timothy strains and also in the timothy and clover fractions as the date of harvest became later. The late timothy was higher in protein than the medium or early timothy at all comparable dates of harvest. Medium and early timothy differed but little in protein content on comparable dates of harvest.

The yield of protein per acre decreased as harvest was delayed; markedly so after the C period. A tendency was noted for the late timothy to exceed the early and medium timothy in yield of protein per acre. This was due in part to the higher percentage of protein in the late timothy fraction and in part to the larger proportion and yield of clover growing with it in the first year's crop.

The percentage of green color and the United States grades of hay produced by the early and the medium timothy were not greatly different at the different harvest periods. At all times the percentage of green color in the hay produced by the late timothy was higher than in the hay produced by either early or medium timothy, and as the season advanced the grade of the late timothy was higher than that of the other two strains.

In northern latitudes, in rotations in which the meadows are maintained 2 or 3 years, late timothy may be preferable to early or medium timothy when farm practices make late harvesting desirable.

When yields of hay, yields of protein, percentages of protein, and grades of hay are all considered, it becomes evident that harvesting when the variety of timothy is in early bloom, or even before, is preferable to harvesting later.

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RESPIRATION AND OXIDASE AND CATALASE ACTIVITY OF APPLE AND PEAR FRUITS[1]

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INTRODUCTION

In connection with certain investigations of apples and pears in storage the question arose as to the relationship between respiratory intensity and oxidase and catalase activity. A review of the literature showed contradictory evidence. In some cases the correlation was strongly positive, in some negative, and in others oxidase, catalase, or respiration preceded, followed, or acted independently of the others.

Since certain disorders of stored fruit, such as scald, soft scald, and bitter pit of apples and loss of ripening capacity in pears, are physiological in nature and may be correlated with, or caused by, respiratory disturbances during storage, a study of the correlation between respiratory intensity and oxidase and catalase activity of apple and pear fruits was made. Numerous determinations of the rate of respiration and of the oxidase and catalase activity were made at Wenatchee, Wash., during 1933 to 1936, under conditions as thoroughly standardized and controlled as possible.

In its broadest sense respiration is a rather involved process of breaking down respirable materials through various stages to the production of the final products (*40*).[2] However, in the present work the writers have used it in a restricted sense to refer only to the final oxidation of the intermediate substances and have taken the carbon dioxide liberated as a measure of the respiratory intensity. Certain enzymes, including oxidase and catalase, may be involved in respiration, and while "respiratory intensity" and "enzyme activity" may not be mutually exclusive, the writers have separated them for the purpose of comparison, to see whether they are always correlated and react similarly to stimuli. Also the term "oxidase," as used by the writers, refers to those enzymes that oxidize various substances directly without the addition of hydrogen peroxide.

Since fruits are living organisms, the rate of respiration is accepted as a measure of their physiological activity. The enzymes oxidase and catalase are sometimes spoken of as respiratory enzymes and are usually assigned an important role in respiration. However, the part they play is largely hypothetical. In the laboratory the oxidases as prepared by present methods do not oxidize the carbohydrates or fats used in respiration. The role of catalase is even more doubtful. Hydrogen peroxide, the substance known to be attacked by catalase, has not been found in the living tissue, although there is evidence that it is produced there (*44*). The wide distribution of catalase and the close correlation of respiratory intensity and catalase activity, espe-

[1] Received for publication July 21, 1937; issued April 1938.
[2] Reference is made by number (italic) to Literature Cited, p. 384.

cially in some of the earlier work, doubtless was a factor in causing investigators to classify catalase as a respiratory enzyme. Later work has not always yielded such conclusive evidence, and in many cases catalase activity has either preceded, followed, or acted independently of respiratory intensity.

REVIEW OF LITERATURE

The literature dealing with the effect of environmental conditions on respiratory intensity and oxidase and catalase activity is so voluminous that it will be necessary to limit this review to certain papers bearing directly on the interrelations of these three systems. For a more complete discussion and bibliography, the reader is referred to Miller (*26*) or to any of the many other excellent reviews and bibliographies dealing with the subject.

Appleman (*1*), working with potatoes, found that where the tubers were treated with ethyl bromide or removed from storage at 3° C. to room temperature, or greened through exposure to light, the respiration rate and catalase activity were greatly accelerated, while the oxidase activity remained practically constant. He also found greater catalase and respiratory activity in the bud half of McCormick tubers, and no difference in the oxidase activity as measured by the Bunzell method; however, colorimetric results obtained by using aloin as the oxidizable substance gave greater oxidase activity in the stem half. Neither method disclosed any correlation between oxidase activity and respiration. There was a large varietal difference in respiration and oxidase activity, Carmen No. 1 respiring more rapidly than McCormick and possessing only one-fourth the oxidase. The catalase activity in the two varieties was strikingly correlated with respiration. These results led Appleman to conclude that in potato tubers there is no correlation between respiration rate and oxidase activity but a striking correlation between respiration rate and catalase activity. The same author (*2*) later found a close correlation between respiratory intensity and catalase activity in sweet corn in the milk stage. In this stage the respiration rate was very high when the corn was first pulled but fell off rapidly with storage. Catalase activity showed a decline with storage proportional to the decline in respiratory intensity. Appleman's work led him to believe that the catalase activity of the expressed juice from both sweet corn and potato tubers was a fair index of the comparative intensity of respiration in the tissues. He states (*2, p. 209*): "The data from both plant and animal tissues available at present seem to justify the general induction that catalase action is invariably correlated with the oxidative processes involved in respiration."

After treating potato tubers with various chemicals to alter the respiration rate, Miller et al. (*28*) found no direct connection between the respiratory rate and catalase and peroxidase activity. Acceleration of enzyme activity followed the increase in output of carbon dioxide, and it occurred after the peak of respiratory activity. Certain chemicals that increased respiration depressed enzyme activity while the respiration rate was most active, but later the enzymes showed greater activity than in the untreated lot. Alcohol decreased respiration but increased the catalase activity. This chemical had a tendency to lower slightly the peroxidase. These investigators reported

also a close correlation between catalase and peroxidase activity, although in certain cases, notably in the alcohol-treated lots, there was a divergence in activity.

Davis (*10*) concluded that blackheart of potatoes was caused by high respiratory activity and a failure of the gaseous exchange to keep pace with the rate of respiration. The catalase activity of affected tissues was not correlated with respiratory intensity, but was more nearly correlated with the oxygen content of the intercellular spaces.

Rhine (*36*) found a large and immediate rise in respiration during the germination of seeds; catalase activity was first decreased and then accelerated. The early curves of respiration and of catalase activity therefore diverged widely. Rhine concluded that catalase could be used as an indicator of metabolism only in cases where there is no rapid change in respiration.

Lantz (*23*) studied the relation between the rate of respiration and the catalase activity during germination at different temperatures of strains of corn varying in chemical composition. In all strains, at all temperatures, catalase activity decreased in the early stages of germination, then increased to a maximum, and finally decreased again as the reserve food of the grain was used. Respiration increased slowly in the early stages of germination and more rapidly in the later stages. Catalase activity at 30° C. did not increase proportionally with the accelerated metabolism caused by the higher temperature. In most cases catalase content at 30° was less than at 20°, and a temperature of 42° markedly reduced the catalase content of corn seedlings. Respiration rose rapidly with an increase in temperature, but catalase failed to show a corresponding increase. Lantz found some correlation between catalase activity and rate of respiration during germination of the different strains of corn at 20°, but no correlation was apparent at 10° and 30°. In general, his results failed to show a close correlation between catalase activity and respiration rate, and he concluded that the evidence did not justify the theory that catalase is the enzyme chiefly concerned in physiological oxidation, but more nearly supports the theory that catalase prevents excessive oxidation.

Becht (*5*) found the blood of animals of the same species, under identical conditions, to vary from 100 to 1,000 percent in catalase activity. He considered catalase activity of doubtful importance in the oxidation process. Morgulis (*29*) found that exposure of frogs to temperatures that affected the metabolic rate from 300 to 400 percent had no influence on the catalase content, and he concluded that whatever the function of catalase might be in the organism it certainly was not a measure of metabolic activity. Seymour (*38*) determined the catalase content of the ventricular muscle of turtle hearts. Hearts with a rapid rate as a result of warming (range 1.5 to 40 times the controls) varied in catalase content both above and below the controls, the greater number showing less catalase.

Bunzell and Kenyon (*7*), working with the growing potato plant, found oxidase activity greater in the tuber than in the foliage of the same plant while the catalase activity was constantly two to four times greater in the foliage. The oxidase activity of the foliage was greatest in the early stages of development, declined with growth, and rose again when growth had ceased, whereas the catalase activity

in each instance behaved in just the opposite way. These investigators suggested a reciprocal relationship between oxidase and catalase activity.

Reed (*35*) concluded that the substances affecting the decomposition of hydrogen peroxide (catalase) are not of necessity concerned with enzymes that accelerate peroxide oxidations (peroxidases or oxidases) in pineapples. He reported no catalase activity in green pineapples and concluded that catalase is not universally present in living cells as Loew and others supposed. Harvey (*20*) also failed to find any catalase in blue-green algae growing in hot springs at a temperature of 65° to 73° C.

Tyson (*43*) has shown catalase activity to be positively correlated with vigor of growth and size of plant in sugar beets and the oxidase activity to be greater in those plants in which the growth was inhibited. Ezell and Crist (*14*) found that the correlation between the activity of oxidase and growth or size of plants was slight, with a tendency to be negative in lettuce, radish, and spinach plants. The correlation of growth or size of plants with catalase activity was better than with oxidase activity and significantly negative.

Gustafson et al. (*16*), working with tomato fruits, concluded that "respiration, growth and catalase activity go hand in hand," although in one set of experiments tomatoes growing more rapidly than 4 percent per day gave less catalase activity than those growing between 2 and 4 percent. Haber (*18*), also working with tomato fruits, studied the relationship of soil reaction, growth, and catalase activity. He used soils varying in pH values from 9 to 4 and found that the neutral soil gave the greatest yield and the lowest catalase activity. Soil reaction had no effect on oxidase activity. Oxidase activity was greater in ripe fruits than in green ones, and catalase activity was greater in green mature fruits than in ripe ones.

De Villiers (*11*) showed an increased catalase activity with increasing maturity in grapes, but when the grapes became fully ripe there was a slight decrease. Oxidase activity and respiration rate decreased with maturity, the curves being almost parallel.

Johnson (*22*) reported the effect of X-rays on *Helianthus annuus*. She found that irradiation of seeds or seedlings caused a decrease in rate of growth, in catalase activity, and in carbon dioxide production (respiration). Oxidase activity was unaffected by irradiation even in dosages heavy enough to cause seedlings from irradiated seeds to die after reaching a height of 1 to 2 cm. Normal and abnormal leaves gave the same oxidase activity.

In work with diseased plants Bunzell (*6*) found that the diseased material had a greater oxidase activity than healthy material. The characteristic symptom of the diseases studied (spinach blight, mosaic of tobacco, leaf curl of potatoes, curly dwarf of potatoes, and curly top of sugar beets) was a stunting of plant growth.

Dexter (*12*) studied the rate of respiration and the catalase and oxidase activity of cabbage and of winter wheat plants during exposure to low temperature (2° C.). He found that the respiration rate of all samples continuously decreased during storage at this temperature, while the oxidase activity appeared unchanged. In cabbage the catalase activity remained unchanged either in the light or in the dark (for 5 days), but in winter wheat it increased in the dark and decreased in the light.

Pope (*33*) reported a negative correlation between respiration rate and catalase activity in homologous younger leaves of comparable barley plants, but since this was contrary to the results of other investigators, he concluded that it was highly probable that any correlation was fortuitous.

Morinaga (*30*) showed that dry seeds of rice contained about one-tenth as much catalase as wheat, barley, oats, or rye. Rice germinating aerobically contained about seven-tenths as much catalase as germinating wheat, oats, or barley. Catalase did not increase in the course of anaerobic germination, but in a medium of reduced oxygen it increased slowly. Hence he concluded that the rate of increase of catalase activity was a function of the free oxygen in the medium. Aerobically grown seedlings with high catalase used much more oxygen than anaerobically grown seedlings with low catalase activity. The anaerobic seedlings gave off a comparatively large amount of carbon dioxide during respiration, whether under normal aerobic or under anaerobic conditions.

Ransom (*34*) found that the catalase activity of water-saturated fruits and seeds of Polygonaceae was greatest at 3° to 9° C. and lowest at 30°, while the rate of respiration was lowest at 3° to 9° and highest at 30°. However, at a given temperature catalase activity and respiration rate were found to be correlated with the rate of afterripening and germination.

Heinicke (*21*) found no consistent correlation between catalase activity and respiration rate in dormant excised apple twigs.

Harding (*19*) found no correlation between catalase activity and respiration rate at 30° and 36° F. in Grimes Golden apples, but at 50° there was a correlation. He reported: "Catalase activity is independent of, and unrelated to temperature." (Respiration rate is usually considered to be directly related to temperature.) "Oxidase and catalase activity were independent of each other, and oxidase was not found to be significant as an indicator in denoting the development of soggy breakdown." Under cold-storage conditions, an increase in catalase activity was a fairly accurate indication of the approach of soggy break-down. Neller (*31*) noted a tendency to a higher catalase content in Jonathan apples going through the breakdown process, and lower activity in the advanced stages of breakdown. In normal fruits that did not develop break-down, the catalase activity increased during early storage and decreased late in storage life. Neller's findings tend to corroborate the theory that catalase may be used as an index of metabolic activity, physiological break-down being associated with, or caused by, an accelerated metabolic rate.

Drain (*13*) reported that the Oldenburg apple was low in catalase as compared with the Winesap apple, although at 25° C. the Oldenburg variety respired about twice as fast as Winesap at the same temperature. Winesap apples held at 25° increased in catalase activity over similar lots held at 0°, but the respiration rate remained practically the same. A lower respiration rate and less oxidase activity were noted in hard ripe fruit than in ripe fruit. Drain concluded that respiration rate and catalase activity were not closely correlated in apple varieties.

Magness and Burroughs (*25, pp. 79–85*) determined the respiration rate and catalase activity of apples after removal from cold storage to

a higher temperature, and found a gradual increase in catalase activity throughout the duration of the test (17 days). Respiration reached a maximum in approximately 1 day. They concluded that catalase followed respiration and was associated with the total respiration that had taken place rather than with the rate at any particular time. Cellar-stored Winesap apples high in catalase had a lower respiration rate when transferred to 65° F. than did fruit from 32° storage low in catalase. Baldwin apples removed from 65° storage, where high catalase activity had developed, showed no decrease in catalase activity after 2 months' storage at 32°, indicating that catalase was not directly related to temperature. Later Magness and Ballard (*24*) found that the highest point in catalase activity came sometime prior to the period of greatest respiratory activity in Bartlett pears stored at 60°. When the pears were almost full ripe, the respiratory activity was increasing while the catalase was decreasing rather rapidly.

Carrick (*8*) found that freezing Winesap apples at −7.5° to −8.5° C. for 3 to 6 hours caused their respiration rate at 0° to increase as much as 85 percent above their previous normal performance at that temperature. This increased rate lasted for several days. With McIntosh apples 4 days after freezing, he found (*9*) the catalase activity lower in the 3-hour treatment but somewhat higher in the 6-hour treatment. With Baldwin apples 3 days after freezing, those frozen for 3 hours did not vary from the controls in catalase activity; those frozen 6 hours showed a slight increase. Seven days after freezing, both the 3- and the 6-hour treated fruits showed a slight increase in catalase activity.

The available experimental evidence is not such as to give conclusive proof that rate of respiration is dependent upon oxidase or catalase activity as measured by present methods. Rather, it raises serious doubt as to whether these activities are directly correlated with the rate of respiration.

MATERIALS AND METHODS

Fruits used in these studies were selected in the orchard for uniformity of growing conditions, size, and other external factors, thereby insuring comparable material for the various phases under investigation. After harvest all lots were treated uniformly except for those variations being studied. When cold storage was used the fruit was stored within a few hours after harvest, and in all cases respiration lots and lots used for enzyme determinations were given comparable treatments.

Rate of respiration was usually determined by enclosing fruit in an airtight container, aspirating with air free from carbon dioxide, and absorbing the respired carbon dioxide in barium hydroxide in Truog absorption towers. Exceptions to this procedure are noted below. Oxidase activity was determined iodometrically as described by Guthrie (*17*). A uniform reaction temperature was obtained by drawing the air through a constant-temperature bath held at 25° C. Enzyme determinations were made in duplicate and if not in close agreement were discarded and samples re-run. Catalase activity was determined by a water-displacement method similar to that described by Heinicke (*21*). Uniform shaking was accomplished by means of a mechanical shaker run by an electric motor geared so that the solution flowed 108 times per minute from one end of the tube to the other.

An even temperature was obtained by immersing the tubes in a thermostatically controlled bath held at 25°. Preliminary tests showed this temperature to give nearly maximum activity with the materials studied.

Balls and Hale (*4*) reported that catalase may exist in the inactive state. Activation of this inactive form could be accomplished by the addition of an activator, such as Kolmer's antigen or an extract prepared from heart tissue, as shown by Swenson.[3] Catalase determinations were made on various samples to which the above-mentioned activators were added. Representative data are given in table 1. The activity reported represents 1 cc of juice plus 1 cc of the activator diluted as indicated.

TABLE 1.—*Effect of activators on catalase in juice from pears*

Activator	Dilution	Activity [1] (O_2 liberated)	Activator	Dilution	Activity [1] (O_2 liberated)	Activator	Dilution	Activity [1] (O_2 liberated)
		Cc			*Cc*			*Cc*
Blank (water)		10.6	Blank (water)		14.7	Heart	0	12.6
Antigen	1 : 100	10.0	Antigen	0	3.3	Do	1 : 100	13.4
Do	1 : 500	10.5	Do	1 : 100	13.2	Heart (no pear juice).	0	.4
Do	1:1,000	9.8	Do	1 : 500	13.1			

[1] Activity of 1 cc of juice plus 1 cc of activator diluted as indicated.

Table 1 shows that the activators did not increase the catalase activity of the fruit juice. This is not surprising, since the juice used had been pressed from the tissue. Swenson and James (*41*) reported inability to separate the inactive catalase from the living cell.

Several methods of taking samples for enzyme determinations have been reported. Most of these include the use of calcium carbonate or other materials to neutralize the acids present in the tissue and thus prevent the destruction of the catalase. In a study of this kind it is of the utmost importance to have a uniform method of taking samples and determining the enzyme activity, while the particular method employed may be of relatively less importance. The methods employed here were adopted for convenience and represent those that would give easily readable differences. Calcium carbonate was used in the preparation of catalase samples, since unneutralized juice gave a very low reading. Rose et al. (*37*) have shown that ions other than hydrogen or hydroxyl may be important in regulating oxidase activity, and that in neutralizing the acids the effect of the salts formed must be taken into account. Since a satisfactory reading could be obtained with the unneutralized juice, no carbonate or other chemicals were added in the preparation of the oxidase samples.

In taking samples for enzyme analyses the fruit was cut through the greatest transverse diameter, thus dividing the seed cavity in half. The seeds were removed and a portion approximately 1 cm thick was taken from each of the cut surfaces, giving a disk from the greatest transverse diameter of the fruit. Ten or more fruits were used for each sample. Sections were taken from the calyx half for catalase measurements. The sections were weighed and 1 percent of calcium carbonate was sprinkled over the cut surfaces. The tissue was then ground in a food chopper with a nut-butter attachment, and

[3] SWENSON, T. L. ROLE OF CATALASE IN BIOLOGICAL OXIDATION AS DEMONSTRATED BY PROTEUS VULGARIS. Thesis, Amer. Univ. (On file in Amer. Univ. Libr., Graduate School.) 1933.

the juice was squeezed through two thicknesses of cheesecloth. It was then filtered through double cheesecloth without pressure, to remove the tissue forced through at the first filtering. The juice was held in the refrigerator at 40° F. for 24 hours before determining the catalase activity. For each determination 1 cc of the neutralized pear juice and 5 cc of dioxygen were used. Since apples are relatively weak in catalase, 5 cc of juice and 5 cc of dioxygen were used. Oxidase samples taken from the stem half were ground, and the juice was extracted as for catalase. Samples were prepared in the forenoon, and enzyme determinations were made in the afternoon usually after the lapse of about 3 hours. The oxidase activity varied widely under the various conditions, and it became necessary to alter the amount of sample in order to insure an excess of oxidizable substance in the substrate. However, the amount of juice used was the same throughout any particular experiment and varied only between experiments.

In presenting the results, respiration is reported as milligrams of carbon dioxide per kilogram-hour. Oxidase is reported as cubic centimeters of N/100 sodium thiosulphate, and catalase as cubic centimeters of oxygen liberated in 5 minutes.

EXPERIMENTAL WORK

RESPIRATION RATE AND ENZYME ACTIVITY OF DELICIOUS APPLES AS INFLUENCED BY EXTERNAL CONDITIONS

The apples used in this experiment were harvested at optimum maturity and stored immediately at 32° F. until ready for use in January. Since certain chemicals are known to affect the rate of respiration in potatoes and other plants, it was decided to treat apples with some of these chemicals and determine the effect on the rate of respiration and the enzyme activity. The substances selected were ethylene and ethylene chlorohydrin to increase respiration and ethyl alcohol to lower it. Preliminary tests showed that when fruit was removed from 32° and placed at 65° the increase in rate of respiration due to the change in temperature alone was so great that differences due to the chemicals were of doubtful significance. Representative rates at 65° were as follows:

Check untreated	19.9 mg CO_2 per kilogram-hour
Ethyl alcohol	18.9 mg CO_2 per kilogram-hour
Ethylene	21.1 mg CO_2 per kilogram-hour
Ethylene chlorohydrin	21.1 mg CO_2 per kilogram-hour

Since the respiration differences were small, no enzyme determinations were made on these lots.

In the tests reported below the apples were treated at 32° F. for 30 hours as follows:

Lot 1.—Check, aerated continuously.

Lot 2.—Aerated with air that had bubbled through 95-percent ethyl alcohol before entering respiration chamber.

Lot 3.—Ethylene, 1 part to 1,000 parts of air, in a closed system with potassium hydroxide as absorbent of carbon dioxide.

Lot 4.—Aerated with air that had bubbled through 40-percent ethylene chlorohydrin before entering respiration chamber.

The results are given in figure 1. The rates of respiration are the average for 95 hours following the beginning of treatment and are representative of differences noted at intervals during the treatment. Enzyme samples were taken at the end of the 95-hour period.

The evidence indicates that the respiration rate is not directly cor-

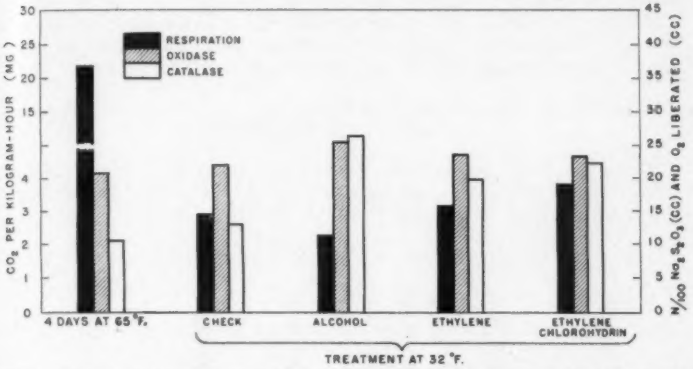

FIGURE 1.—Respiration rate and enzyme activity of Delicious apples as influenced by external conditions.

related with oxidase or catalase activity in cold-stored Delicious apples. The catalase and oxidase also acted independently of each other. The rate of respiration was not affected as much by the chemicals as has been previously reported with potatoes; however, alcohol caused a depressing effect and ethylene and ethylene chlorohydrin an accelerating effect. Both oxidase and catalase activity were greater in the chemically treated fruit than in the untreated check. Catalase activity was from 50 to 100 percent greater in the treated lots, irrespective of whether the respiration rate was increased or depressed. Alcohol, which had a depressing effect on respiration, caused a greater increase in enzyme activity than did ethylene or ethylene chlorohydrin.

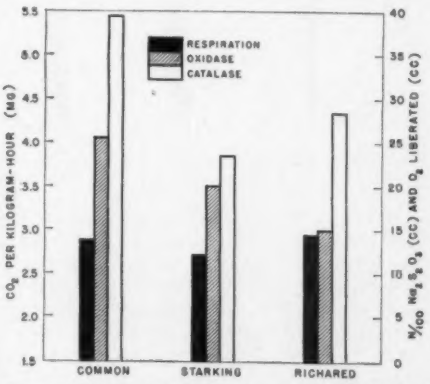

FIGURE 2.—Respiration rate and enzyme activity of different strains of Delicious apples.

Upon the termination of these experiments the check lot of fruit was removed from 32° to 65° F. for 4 days; the respiratory intensity increased 676 percent, while the oxidase and catalase activity decreased slightly. At the end of 8 days at 65° the oxidase activity was still lower, but the catalase activity had more than doubled.

RESPIRATION RATE AND ENZYME ACTIVITY OF STRAINS OF DELICIOUS APPLES

Determinations of rates of respiration and of oxidase and catalase activity were made on three strains of Delicious apples (common Delicious, Starking, and Richared). The fruits were stored at 32° F. immediately after harvest, and determinations were made 36 hours later. The results are given in figure 2.

There is no correlation evident between oxidase and catalase activity and rate of respiration in different strains of Delicious apples. Oxidase and catalase also act independently of each other.

RESPIRATION RATE AND ENZYME ACTIVITY OF ROME BEAUTY AND WINESAP APPLES AS INFLUENCED BY TEMPERATURE

Respiration rate and oxidase and catalase activity were determined on Rome Beauty and Winesap apples at harvest and after storage at 65° and at 32° F. The results are given in figure 3.

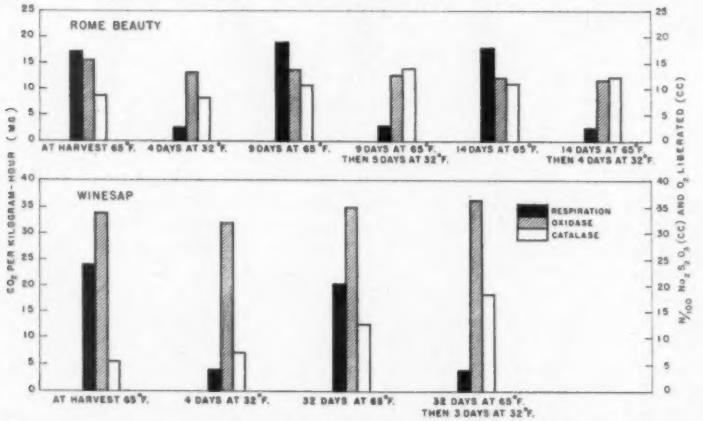
ROME BEAUTY
WINESAP
RESPIRATION
OXIDASE
CATALASE
AT HARVEST 65°F.
4 DAYS AT 32°F.
9 DAYS AT 65°F.
9 DAYS AT 65°F. THEN 5 DAYS AT 32°F.
14 DAYS AT 65°F.
14 DAYS AT 65°F. THEN 4 DAYS AT 32°F.
32 DAYS AT 65°F.
32 DAYS AT 65°F. THEN 3 DAYS AT 32°F.
CO2 PER KILOGRAM-HOUR (MG.)
N/100 Na2S2O3 (CC) AND O2 LIBERATED (CC)

FIGURE 3.—Respiration rate and enzyme activity of Rome Beauty and Winesap apples as influenced by temperature.

With Rome Beauty apples under the different storage treatments the respiration rate ranged from 2.45 to 18.90 mg of carbon dioxide per kilogram-hour, an increase of 671 percent. The oxidase activity ranged from 11.5 to 15.6 cc, an increase of 35 percent. The respiration rate rose during storage at 65° F., reaching a climax in 9 days. Oxidase activity declined slowly but steadily at this temperature. At 32° the respiration rate dropped to approximately 15 percent of its intensity at 65°. There was only a slight decrease in oxidase activity, while in certain cases catalase was greater. In Winesap apples the respiration rate and oxidase and catalase activity showed a similar tendency to act independently of each other.

RESPIRATION RATE AND ENZYME ACTIVITY OF ROME BEAUTY APPLES AS INFLUENCED BY RESPIRATORY STIMULANTS FOLLOWING HARVEST

It was thought that apples treated immediately after harvest while in a preclimacteric respiratory stage might be more subject to respira-

tory stimulants than lots that had been stored at 32° F. until they reached the postclimacteric stage and had then been brought to the warm room. In the latter case it had been found that changes due to the sudden rise in temperature from 32° to 65° largely obliterated the effect of the stimulants. Rome Beauty apples were brought to the 65° room immediately after harvest and subjected to the following treatments:

Lot 1.—Check, aerated continuously.
Lot 2.—Aerated with air that had bubbled through 40-percent ethylene chlorohydrin before entering respiration chamber.
Lot 3.—Aerated with air mixed 50–50 with oxygen gas before entering respiration chamber.

In order to determine the cumulative effect of the gases, the treatment was continued for 12 days and was followed by 5 days' aeration without the gases. The results are given in table 2.

TABLE 2.—*Respiration rate and enzyme activity of Rome Beauty apples as influenced by respiratory stimulants immediately following harvest*

Storage at 65° F. (days)	Check			Ethylene chlorhydrin [1]			Oxygen [1]		
	Respiration rate (CO_2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)	Respiration rate (CO_2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)	Respiration rate (CO_2 per kg-hr.)	Oxidase activity (N/100 $Na_2S_2O_3$)	Catalase activity (O_2 liberated)
	Mg	*Cc*	*Cc*	*Mg*	*Cc*	*Cc*	*Mg*	*Cc*	*Cc*
1	14.05			20.25			15.84		
2	16.06			21.63			20.57		
3	17.01	9.2	8.1	21.11	7.2	6.7	20.98	6.8	7.8
4	18.36			21.45			22.69		
5	19.52			21.67			23.37		
6							23.07		
7	18.94			20.87			21.74		
8	18.15								
9	18.22			20.56			21.11		
11	18.68			20.87			21.46		
12		5.6	12.9		6.1	6.8		6.6	13.2
14				19.80			18.12		
16	16.37			21.39			18.23		
17	17.91			22.41			18.82		
18		6.5	12.2		6.4	6.3		6.5	12.7
19	15.82								

[1] Treatment discontinued after 12 days.

While the gases caused an appreciable increase in respiration, the rate of respiration and oxidase and catalase activity were not parallel in Rome Beauty apples subjected to respiratory stimulants immediately after harvest. Ethylene chlorohydrin caused a rapid rise in respiration, which continued beyond the period of treatment. It is possible that the treatment was too severe for the best results. The respiration rate rose rapidly and remained practically stationary for several days; it decreased slightly and then rose above the previous maximum after the treatment was discontinued. Ethylene chlorohydrin could be tasted in the fruit for several days. No visible injury was evident at the end of the treatment, but 6 days later, when the last enzyme determinations were made, approximately one-third of the fruits were showing injury at the core. It is evident that the vapors had been interfering with the catalase activity much earlier, possibly within 3 days.

The apples responded a little more slowly to oxygen gas than to ethylene chlorohydrin vapor. Apparently the increased concentration of oxygen did not interfere with the normal metabolism of the fruit as much as did chlorohydrin. The rate of respiration was higher in the oxygen-treated lot than in the check lot, but otherwise the behavior was much the same. Both lots reached the respiratory climax in 5 days. At the end of 3 days the oxidase activity was lower in the treated lot, but it was approximately the same in both lots at the end of the treatment and again 6 days later. Catalase activity of the two lots was closely parallel during the test.

RESPIRATION RATE AND ENZYME ACTIVITY OF BARTLETT PEARS

Since it is known that very immature fruit respires much more rapidly than mature fruit and also that oxidase and catalase activity are greater in immature fruit, a study was made of the changes in these activities in Bartlett pears during the growing season. The first determinations were made June 11, when the average weight per fruit was 19.1 g, and at intervals thereafter until September 9, when the average weight per fruit was 270.5 g. The last sampling was far past the normal harvest season. The fruit was picked between 8 and 9 a.m. each time throughout the season at several points in the same tree, so as to get a representative sample. The tree was located on a moderately heavy clay loam, was in vigorous growing condition, and bore a good crop of fruit. Immediately after harvest the fruit was taken to the laboratory and the samples were prepared. Fruit used for determining respiratory intensity was placed in airtight containers, and determinations were made in the afternoon of the day the fruit was picked. Oxidase and catalase samples were prepared from comparable lots of fruit in the forenoon of the same day. The oxidase activity was so great in the very immature fruit that it was necessary to reduce the amount of juice taken for analysis from 10 cc, as previously reported, to 1 cc. Pear fruits in general are much richer in catalase than apple fruits, and 1 cc of juice was also used for the catalase determinations. The results are given in figure 4.

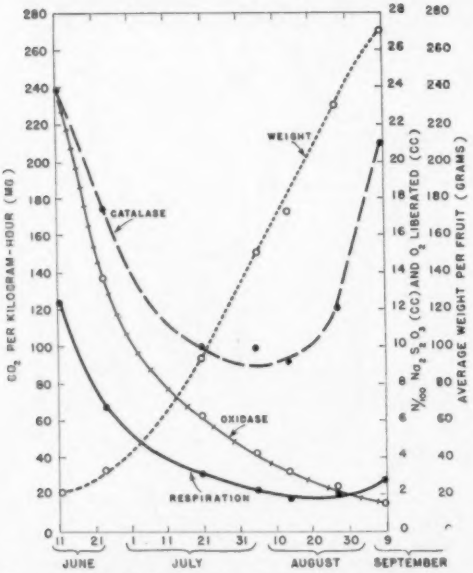

FIGURE 4.—Respiration rate and enzyme activity of Bartlett pears during the growing season.

Figure 4 shows a consistent increase in the size of the fruit throughout the growing season. The rate of respiration and oxidase and catalase activity showed a corresponding decrease until August 14, at which time the fruit showed a pressure test of 17.9 pounds, which is usually recommended for the harvesting of Bartlett pears for best dessert and storage quality. Oxidase activity continued to decrease as long as the fruit remained on the tree, but the rate of respiration and catalase activity increased after the August 14 minimum. If determinations had been made at shorter intervals immediately preceding and following this date a lower minimum might have been recorded, but it is of interest to note that the minimum in both respiration and catalase activity came at the time when experience had showed that the fruit should be harvested. A strong positive correlation is evident between the rate of respiration and catalase throughout the growing season. Oxidase activity also followed closely until the fruit was ready for harvest, but if the fruit was allowed to remain on the tree until it was "tree ripe" the oxidase activity decreased further while catalase activity and the rate of respiration increased.

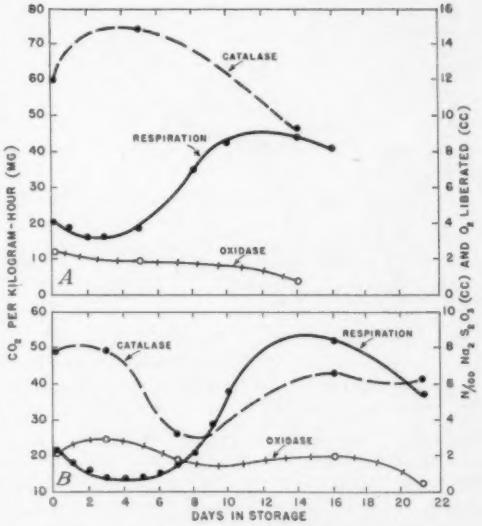

FIGURE 5.—Respiration rate and enzyme activity of Bartlett pears during ripening at 65° F.: *A*, Mature fruit; *B*, immature fruit.

This correlation between rate of respiration and enzyme activity was evident only at harvest. When fruit was held in storage for several days the rate of respiration and enzyme activity diverged. This divergence, shown in figures 5 and 6, held true whether the fruit was harvested while still immature or was permitted to reach full maturity.

In connection with the rise in respiration on removal of the fruit from 32° to 65° F., as shown in figure 6, it should be noted that the rate at the end of the first day at the higher temperature was greater than at the end of the second day. Willaman and Brown (*45*) attribute this rise to a lower carbon dioxide solubility in the cell sap at the higher temperature, with consequent release of the excess gas.

RESPIRATION RATE AND ENZYME ACTIVITY OF ANJOU PEARS DURING STORAGE

Anjou pears were harvested at optimum maturity (11.6 pounds pressure) and stored immediately at 32° F. The rate of respiration and enzyme activity were followed during the storage season. The results are given in figure 7.

It is evident that the rate of respiration is not correlated with enzyme activity in Anjou pears stored at 32° F. The rate of respiration fluctuated during the storage season, but no definite trend was noticeable. Oxidase activity increased steadily, more than doubling during the season. Catalase activity also increased but fluctuated more widely than oxidase. Other varieties gave similar results. Unpublished data have shown that within the commercial picking range the maturity of the fruit at harvest exerts little influence on

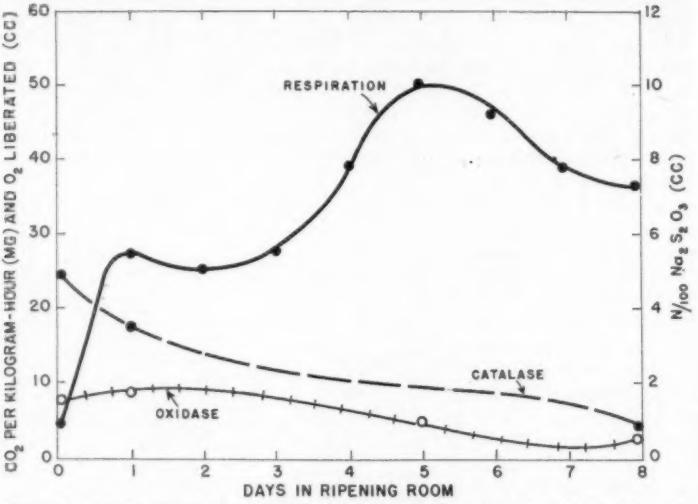

FIGURE 6.—Respiration rate and enzyme activity of Bartlett pears stored at 32° F. for 14 days and ripened at 65°.

the rate of respiration at 32° but exerts considerable influence on the oxidase activity. With the less mature fruit the oxidase activity increases rapidly with time in storage, but with more mature fruit the increase is less.

RESPIRATION RATE AND ENZYME ACTIVITY OF BOSC PEARS FROM DIFFERENT LOCALITIES

Bosc pears grown at Wenatchee and Yakima, Wash., and at Medford, Oreg., were harvested and stored at 30° and 36° F. within 24 hours after harvest. Rates of respiration and oxidase and catalase activity were determined after 3 months' storage. The results are given in figure 8.

The rates of respiration were practically the same in the fruit from the three districts. The averages of the enzyme values from the two temperatures show that the Medford fruit contained about five and one-half times as much oxidase as the Wenatchee fruit and over three times as much as the Yakima fruit. Medford Bosc pears also contained more than twice as much catalase as Wenatchee Bosc and nearly twice as much as Yakima Bosc. At 30° F. the average output of carbon dioxide was about two-thirds of that at 36°. The oxidase activity did not vary greatly, and the catalase activity at 30° was approximately two-thirds of that at the higher temperature.

DISCUSSION

Throughout the work it was evident that the chemical stimulants used were not so effective with apples and pears as has been reported with potatoes. Miller and Denny (*27*) have shown that the amount of ethylene chlorhydrin taken up by potato tubers varies with the permeability of the skin. The skin of apple fruits is comparatively tough and has a coating of natural wax. This skin doubtless is

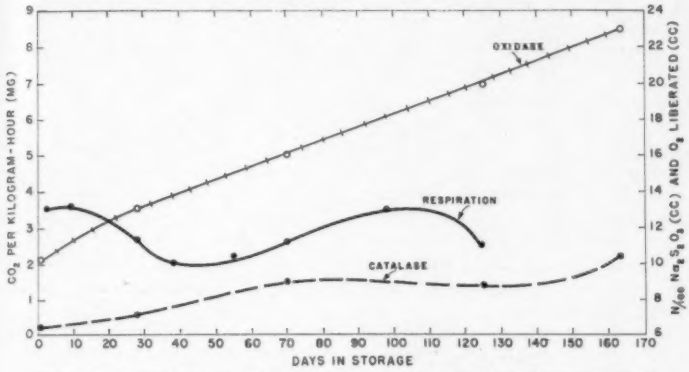

FIGURE 7.—Respiration rate and enzyme activity of Anjou pears during storage at 32° F.

much less permeable than the least permeable potato skin. Pear skins are less tough than apple skins and comparatively free from wax. Gerhardt and Ezell (*15*) have shown that pears absorb gas much more readily than apples, as measured by the internal concentrations of carbon dioxide after the fruit has been subjected to the gas for a definite period. On removal to normal atmospheres

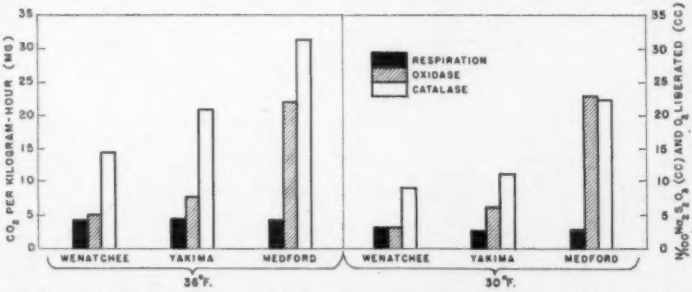

FIGURE 8.—Respiration rate and enzyme activity of Bosc pears from the Wenatchee, Yakima, and Medford districts, stored at 30° and 36° F.

pears also lost the gas more rapidly and reached equilibrium at about the same time as apples, indicating that pears are more permeable to gases than are apples. However, Anjou pears held at 32° F. for 2 months and then treated with ethylene and ethylene chlorohydrin for 48 hours at 32° showed no significant increase in respiration over the check. When treated at 65° for 24 hours no significant differences were noted during treatment or during subsequent ripening.

From the results reported herein it appears that apple and pear fruits are much less susceptible to chemical respiratory stimulants than are potatoes. However, it is well to remember that they represent entirely different types of tissue. Mature fruits, exclusive of seeds, consist of mature cells and are incapable of further growth or division. Potatoes are fleshy underground stems composed largely of storage cells and meristematic tissue in the resting stage. Under favorable conditions they are capable of growth, production of new cells, and the completion of the life cycle, all of which necessitate increased respiration.

The results herein presented show that the intensity of respiration and the activity of the so-called respiratory enzymes, oxidase and catalase, are apparently not closely correlated in apple and pear fruits when held in storage. The data show that the enzymes act independently of the rate of respiration and independently of each other. However, in Bartlett pears sampled immediately after picking there was a close correlation between respiration and catalase activity throughout the growing season. Oxidase activity likewise was closely correlated with respiration until the fruit was ready for harvest, but if the fruit was allowed to remain until "tree-ripe", the oxidase activity decreased while the rate of respiration and catalase activity increased.

Since the status of oxidase and catalase as respiratory enzymes is largely hypothetical, with little or no direct evidence to support the theory that they are responsible for respiratory intensity, and since results of previous work show such wide divergence, one might be inclined to question the part that these enzymes play in respiration. Perhaps the correlations found have been fortuitous and without special significance.

Another interpretation, and one that seems more logical, is to accept oxidase and catalase as respiratory enzymes and credit them with accelerating certain chemical changes that take place during respiration. Certainly it is necessary to assume the presence of some activating substance in the living cell to account for the ease and rapidity with which chemical reactions take place and compounds are broken down to release energy for the cell. However, it has been pointed out (*14, p. 21*) that—

> there is no evidence to support any theory which would embody the concept of there being an exact quantitative balance between the necessary amount of energy and the activity of energy-producing agencies. This would attribute to plant organisms a degree of economy and an inherent capacity for conservation of energy which they may or may not and most likely do not possess.

The above concept of the interrelationship between respiration and oxidase and catalase activity need not conflict with the findings of other workers. Instead it offers a possible explanation of some apparent discrepancies. Oxidase and catalase enzymes could undoubtedly be present in excess in tissues and bear a relation to respiration comparable to that of the large reserves of food materials, none of which would be likely to become limiting under normal conditions. Seeds containing large reserves of stored food respire very slowly until they start to germinate, and the seedlings increase in respiratory intensity to a maximum and then decline; the curve of this process is practically identical with the grand curve of growth (*32, p. 217*). Thus the low points in the respiration curve are at the beginning and

at the end of the life of the plant, at which times the amount of stored food is at its maximum. Miller discusses the effect of food supply on the rate of respiration, and states (*26, p. 733*): "The total quantity of carbohydrate in the cell will thus not influence the rate of respiration except to determine the length of time that the process may continue therein." More recently Appleman and Smith (*3*) have shown that there is no direct correlation between the rate of respiration and the total or reducing sugar content. Possibly nature is not so economical with respiratory enzymes that there would be a supply sufficient to take care of immediate needs only, when there is normally an abundance of reserve materials present in excess of the amount consumed in respiration.

If the foregoing hypothesis be true and the intensity of respiration increases rapidly, as in germinating seed, the enzymes present are sufficient to take care of the increase in respiration, although if the enzyme content is relatively small it may decrease until an additional amount can be produced; this would explain the delayed rise in catalase activity as reported by Rhine (*36*), Lantz (*23*), and Miller et al. (*28*). If the supply of the enzyme is relatively large, as in fruits, then the enzyme activity may increase slowly as additional catalase is produced; and the enzyme activity may follow the rate of respiration, as reported by Magness and Burroughs (*25*), or decrease if conditions are unfavorable for production of the enzyme, as reported by De Villiers (*11*).

That the catalase should increase as the fruit ripens on the tree (fig. 4) but decrease as it ripens in the ripening room (figs. 5 and 6) may seem rather contradictory on first thought. Two explanations may be offered.

(1) The type of ripening is different in the two cases. Bartlett pears harvested at commercial maturity and ripened at 65° F. become soft and juicy with almost a buttery texture. The fruit becomes increasingly soft as it approaches the overripe stage, and the tissue throughout finally collapses. However, fruit allowed to ripen on the tree remains firm and crisp and the texture becomes granular. As maturity advances break-down begins at the core and progresses outward, leaving only a shell of apparently sound tissue filled with broken-down cells, the shell finally collapsing. In these two types of ripening, apparently dependent on different factors during the ripening process, the need for catalase may not be the same.

(2) When fruit is harvested at commercial maturity and ripened at 65° F., all the processes of ripening are katabolic. The products of decomposition may accumulate rapidly to injurious proportions and destroy the catalase. Fruit previously stored at 32° ripens quickly, decomposition products accumulate rapidly, and a continuous decline in catalase may be observed (fig. 6). When fruit is allowed to ripen on the tree both anabolic and katabolic processes are active, the relative importance of the two changing as the season advances. The products of katabolism in small amounts may then act as a stimulus to catalase activity, with the resultant U-shaped curve (fig. 4), which if prolonged until decomposition is well advanced would doubtless be S-shaped. Evidence that small amounts of decomposition products of ripening fruit may stimulate catalase activity is seen in figure 5. With mature fruit (fig. 5, *A*) visible ripening starts within a few days and catalase increases and then declines as decomposition products

accumulate. With immature fruit (fig. 5, *B*) visible ripening does not take place for several days and catalase decreases, and then increases as visible ripening begins with the resultant stimulatory action. If fruit is stored at 32°, decomposition products should be formed slowly, and catalase should increase slowly, as is shown in figure 7. That alcohol and ethylene chlorhydrin in small amounts may act as a stimulus to catalase activity has been shown (fig. 1). Table 2 shows that if the treatment is prolonged catalase activity will be lowered. Alcohol and acetaldehyde increase in fruit as it ripens, reaching a maximum in the overripe fruit. Fruit with a high acetaldehyde content has been found to be uniformly low in catalase.

Bunzell and Kenyon (*7*) have considered a possible reciprocal relationship between oxidase and catalase activity. They have advanced the theory that a portion of the colloidal constituents, such as proteins, may be responsible for the oxidase activity. A different type or degree of colloidality may be responsible for catalase activity, and the two types may be so interrelated that each tends to operate to the exclusion of the other. A reciprocal relationship doubtless sometimes occurs; however, if this relationship held universally, then the two enzymes would not parallel each other as Dexter (*12*) found with cabbage plants exposed to low temperature or as the present writers found with Bartlett pears during the growing season (p. 377). In certain cases conditions may be more favorable for the production of one type of enzyme than the other and a reciprocal relationship may appear to exist. If catalase and oxidase catalyze different reactions, then those conditions in which more of one enzyme would be needed may not necessarily be the same as for the other. Thus in spinach blight (*6*) additional oxidase may be produced by the plant, while with a different type of disease, such as soggy break-down of apple fruits (*19*), additional catalase may be produced. Until more is known about the part enzymes play in the cell and the reaction that each catalyzes, the reason for their increase will probably remain unknown.

In connection with the respiration-oxidase-catalase relationship, the work of Balls and Hale (*4*) is of interest. In 1932 they reported the discovery of an inactive form of catalase that could be activated by the addition of S–S derivatives (cystine, insulin, and oxidized glutathione). The S–S-containing compounds may be replaced by heart-muscle extract as reported by Swenson.[4] Swenson interpreted the presence of the inactive form as explaining the contradictory results of previous workers who attempted to correlate catalase activity with metabolic processes but failed to consider the temporarily inactive form.

With the addition of activators, Swenson found that the previously inactive form was like the original active form in being able to liberate oxygen from hydrogen peroxide but apparently differed from it in other respects. Thus, he found that there were either two forms of inactive catalase or, less probably, that two activators were necessary for its activation. Also, the inactive part is destroyed when the cell is killed by acetone, even though the activation takes place before the death of the cell. The original active form remains active after the cell is killed, but the recently activated form disappears. Working with bacteria, Swenson concluded that the difference was probably due to

[4] SWENSON, T. L. See footnote 3.

the manner in which the enzyme was attached to the cell. He found that the catalase content of bacteria varied with the oxygen supply and probably depended on the rate of cell oxidation. Most of the increased activity was of the inactive type. The increased catalase with the higher rate of oxidation lends support to the catalase-respiration relationship.

In interpreting the work of Swenson in relation to the hypothesis presented in this paper, it might be better to visualize the inactive catalase of Balls and Hale as a "proenzyme" (*42, p. 198*), i. e., catalase in various stages of development. At some stage in its formation, if an activator is added, it is able to decompose hydrogen peroxide in oxygen and water, although it is not fully stable catalase that can be extracted from the cell and treated as normally active catalase. This conception would be analogous, as mentioned above, to reserve food stored in a cell or plant. There is enough available food present normally to supply the immediate needs of the plant, but if an extra drain is made on it, as in germinating seeds, the reserve food in various forms is rapidly changed to a form that may be used in respiration; likewise, inactive catalase may exist as a proenzyme, and its availability for use may depend upon the needs of the plant or the presence of certain activators. Where the cell, as in bacteria, is exposed directly to the stimulant, oxygen, its response is very marked as evidenced by the rapid increase of the inactive catalase. The active catalase increased less rapidly, but probably fast enough to take care of any needs that were likely to develop.

Assuming that the reaction between activators and inactive catalase is a chemical one—and this conforms to modern enzyme theory—the efficiency of different activators would depend on the ability of the activator to join with the inactive catalase molecule and produce the active form. Thus Kolmer antigen would be able to supply more of the active catalase molecule in *Bacillus vulgaris* (Hauser) Migula (*Proteus vulgaris* Hauser) than would heart extract, i. e., it would activate more of the potential catalase in its various stages of formation and produce greater activity. If this explanation of activators is the correct one, it seems unlikely that there is any close correlation between catalase activity, including the inactive portion, and respiratory intensity, as proposed by Swenson. The measurement of the inactive form would depend upon the amount activated from the various stages of formation, and has been shown [5] to vary from one activator to another (Kolmer's antigen was more effective than heart activator); from one treatment to another (at 25° and 37° C. activation took place in 10 minutes' exposure, but at 0° it did not take place in 60 minutes' exposure); and from one organism to another (cystine for liver, heart extract for bacteria). Consequently, until the total potential catalase can be activated under various conditions and with various activators, it will be difficult to correlate respiratory intensity with the summation of the active and inactive forms. Otherwise the discovery of a more efficient activator or a more nearly perfect set of conditions for activation might completely invalidate the data secured. Since there is no evidence to support the theory that "inactive catalase" can function in the cell as active catalase or is available for use until changed to the active form, it seemed preferable to confine the work largely to the active form of catalase.

[5] SWENSON, T. L. See footnote 3.

The recent work of Stern (*39*) is of major interest to students of enzymology because of the direct evidence that he has obtained of the combination of the enzyme with the substrate. When monoethyl hydrogen peroxide is added to liver catalase the enzyme spectrum disappears and two new absorption bands appear. In a few minutes the bands disappear and the original enzyme spectrum reappears. The reappearance of the enzyme spectrum coincides with the disappearance of the titratable peroxide oxygen. No gas is evolved in the reaction. So far as the writers are aware, this is the first direct evidence not only of a combination of enzyme and substrate, but also of a combination between catalase and monoethyl hydrogen peroxide with the liberation of nascent oxygen. Molecular oxygen from hydrogen peroxide is usually assumed to be the normal catalase reaction. No change in the enzyme spectrum was observed when hydrogen peroxide was used as a substrate, the reaction probably being too rapid to be detected. If future work proves that catalase may liberate nascent oxygen in the living plant the present conception of plant respiration and catalase activity may have to be altered substantially.

SUMMARY

The oxidase and catalase activity of apple and pear fruits under various conditions has been studied in relation to the rate of respiration and to each other.

Oxidase and catalase activity were not directly correlated with rate of respiration or with each other, in fruit subjected to various storage temperatures or to various chemical respiratory stimulants or depressants.

There was a positive correlation between rate of respiration and oxidase and catalase activity in Bartlett pear fruits on the tree from the time they were very small until they reached commercial harvest maturity. When the fruits were permitted to remain on the tree past the normal season, the oxidase activity continued to decline while the rate of respiration and catalase activity increased.

There was a positive correlation between catalase activity and rate of respiration in Bartlett pear fruits as long as they remained on the tree.

The intercorrelation of respiration, catalase, and oxidase failed to hold after the fruit was removed from the tree and placed in storage. This was true whether the fruit was harvested relatively immature or whether it was allowed to reach full harvest maturity.

Chemical respiratory stimulants were not so effective with apple and pear fruits as has been reported with potatoes.

The interrelationship of oxidase, catalase, and rate of respiration is discussed. A possible explanation is suggested for the apparently conflicting results obtained by workers in this field.

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THE INFLUENCE OF VARIOUS FACTORS ON EGG PRODUCTION IN TURKEYS [1]

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INTRODUCTION

It has long been recognized that hereditary as well as environmental factors influence egg production in the domestic fowl. Following the work of Pearl (*9*),[3] Goodale (*2*) and later Hays (*3*) established the existence of several inherited factors, each influencing egg production. These factors include sexual maturity, winter pause, broodiness, persistency, and rate of production. Sexual maturity in chickens has usually been measured by age at first egg; winter pause by a period of nonproduction of 7 (or 4 days, Hays (*5*)) days or longer; broodiness by actual cessation of egg production due to the hen's becoming broody; persistency by the length of the laying year; and rate of production by a variety of measures such as percent production for the year or for a given period. These factors have been regarded as independent of each other except sexual maturity and persistency, which Hays (*4*) considers to be linked. Lerner and Taylor (*6*) have shown, however, that persistency as defined by Hays is determined partly by age at first egg, in which case the relation claimed by him would not hold. They used age at last egg as the measure of persistency, and found that, when the term is so defined, there is no evidence of linkage between persistency and sexual maturity. The object of the present paper is to determine how certain factors known to influence egg production in chickens may affect egg production in turkeys.

MATERIALS AND METHODS

The first-year records obtained by trap nesting all the turkeys in the breeding flock during a 3-year period were used. The total number of these records was as follows: For 1934, 91; for 1935, 74; and for 1936, 73. Of these, all the 1934 records were for birds of the Bronze variety; but the 1935 and 1936 records include a few for Bourbon Reds, Narragansetts, and cross-breds. The birds were hatched in April and May. None of them laid before January 1 of the following year. All had completed their first-year record, and all were apparently normal. Selection was not practiced in any way except to exclude a few birds that became sick or died.

The various measures of the factors to be considered were calculated from the trap nest and other records. These include date of hatch, calculated as the number of days from the first of the year in

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[2] Dr. I. M. Lerner and Dr. L. W. Taylor kindly permitted the writer to refer to an unpublished manuscript of theirs. Dr. Lerner also rendered valuable assistance in connection with tables 2 and 3 which were partly calculated under the auspices of the Works Progress Administration.

[3] Reference is made by number (italic) to Literature Cited, p. 392.

which the bird was hatched; age at first egg, in days; date of first egg calculated as the number of days from the first of the year in which the record was made (in all cases the year following the one in which the bird was hatched); age at last egg, in days; date at last egg, in days from the first of the year in which the record was made; length of laying year, in days (= date of last egg minus date of first egg plus 1); length of all pauses [4] of 7 or more days; number of all pauses of 7 or more days; net annual rate measured by the number of eggs laid divided by the length of laying year less the number of days in all pauses of 7 days or more; net spring rate similarly calculated on the basis of eggs laid in March and April. The choice of a 7-day period of nonproduction as the minimum period recognized as a pause, was purely arbitrary. Marsden (*8*) has also, apparently arbitrarily, selected 5 days as the minimum period. No attempt was made to determine the best methods of calculating rate or the best measure of rate. March and April were, however, chosen for the spring period because rate of production, as calculated, was higher in these than in other months. Table 1 presents the means and standard deviations for first year egg production and for each of the factors correlated with first year egg production.

Coefficients of correlation were calculated by the methods described by Ezekiel (*1*) except for coefficients of multiple correlation, which were calculated by the method of Wallace and Snedecor (*11*).

TABLE 1.—*Mean and standard deviation for first-year egg production and the various factors correlated therewith*

Factor	1934		1935		1936	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
First-year egg production.....number..	77.2	30.9	76.1	30.3	79.4	29.8
Date hatched [1].....days..	124.9	14.2	111.7	14.7	115.4	14.1
Date of first egg [1].....do....	57.8	14.5	55.2	17.3	53.7	16.0
Age at first egg.....do....	296.9	18.8	308.3	20.7	303.0	29.3
Date of last egg [1].....do....	223.2	49.8	215.4	39.8	215.7	42.6
Age at last egg.....do....	464.7	54.4	471.1	46.2	464.9	42.7
Length of laying year.....do....	161.6	55.7	163.1	48.0	161.3	45.3
Total length of pauses.....do....	41.3	35.0	39.6	27.7	37.6	32.1
Pauses.....number..	4.4	1.7	2.3	1.6	2.1	1.4
Annual net rate.....percent..	61.4	6.8	61.3	8.5	64.6	7.3
Spring net rate.....do....	71.1	5.8	68.8	9.6	69.1	9.6

[1] Date is expressed in each case as the number of days from December 31. Thus the average date hatched was May 5 (124.9 days), April 22 (111.7 days), and April 25 (115.4 days).

STATISTICAL ANALYSIS

The zero order coefficients of correlation (r) are presented in table 2 together with $\bar{r}$, the coefficient most probably representing the true value for the universe from which the sample was drawn. (See Ezekiel (*1*).) The objects of presenting the data in this table are: (1) To determine which of the factors considered influence annual egg production; (2) to determine which measure should be used when more than one measure of a factor is available (for example, date of first egg and age at first egg as measures of sexual maturity); (3) to

[4] The word "pause", as used herein, includes broody and nonbroody periods. The majority of the birds that paused did not go broody.

determine whether the factors correlated with egg production are correlated with, or are independent of, each other.

Date of hatch has, apparently, no consistent influence on egg production. This conclusion would probably not apply to birds hatched late in the season. Of the two measures of sexual maturity, date of first egg is more closely correlated with egg production than is age at first egg. Date of first egg would seem, therefore, to be the more suitable measure of sexual maturity. Date of last egg and age at last egg are both highly and about equally correlated with egg production. Date of last egg has therefore been selected as the measure of persistency to be uniform with the measure of sexual maturity (date of first egg).

TABLE 2.—*Zero order coefficients of correlation*

Variables correlated	1934		1935		1936	
	r	$\bar{r}$	r	$\bar{r}$	r	$\bar{r}$
First-year production; date hatched	−0.229	−0.205	−0.339	−0.320	0.205	+0.169
First-year production; date of first egg	−.460	−.450	−.518	−.508	−.311	−.290
First-year production; age at first egg	−.170	−.134	−.193	−.154	−.367	−.350
First-year production; date of last egg	.654	.649	.648	.642	.572	.564
First-year production; age at last egg	.679	.675	.667	.661	.520	.510
First-year production; length of laying year	.715	.711	.726	.721	.681	.675
First-year production; length of pauses	−.212	−.186	−.327	−.307	−.395	−.380
First-year production; number of pauses	.059	0	−.071	0	−.060	0
First-year production; annual rate	−.104	0	.273	.248	.176	.132
First-year production; spring rate	.263	.242	.401	.386	.337	.318
Length of pauses; date of first egg	−.025	0	.063	0	.125	.042
Length of pauses; date of last egg	.475	.466	.357	.340	.410	.395
Length of pauses; length of laying year	.452	.442	.259	.233	.320	.300
Length of pauses; spring rate	.105	0	.187	−.147	−.011	0
Spring rate; date of first egg	−.012	0	.117	0	.100	0
Spring rate; date of last egg	.168	.132	.159	.108	.233	.202
Spring rate; length of laying year	.138	.028	.028	0	.155	.101
Spring rate; annual rate	.587	.581	.773	.756	.679	.673
Length of laying year; date of first egg	−.381	−.368	−.659	−.653	−.262	−.236
Length of laying year; date of last egg	.963	.963	.888	.886	.913	.912
Date of first egg; date of last egg	−.127	−.071	−.226	−.195	.088	0

Length of laying year is more closely correlated with egg production than date of last egg. As pointed out by Lerner and Taylor (*6*), length of laying year depends upon age at (or date of) first and last egg, which in the present work, in complete agreement with their data for chickens, are independent variables (table 2). That date of first egg and date of last egg determine the length of the laying year is shown by the coefficients of multiple correlation for these three variables, which ranged from 0.976 to 1.000. Length of laying year, therefore, cannot logically be used as a measure of persistency.

Length of pauses is correlated with egg production, whereas number of pauses is not. Length of pauses is influenced by date of last egg. There is also a significant correlation between length of pause and length of laying year, but this obviously results from the close association between the latter and the date of last egg.

Net rate for the year is not correlated with egg production, whereas there is a significant correlation between egg production and net spring rate. Spring rate and annual rate, though correlated, appear to be independent of other variables, except, perhaps, date of last egg.

The data in table 1 show that sexual maturity (date of first egg), persistency (date of last egg), pauses (length of pauses), and rate (net spring rate) influence egg production. As measured by the coefficient of simple correlation, date of last egg is the most important of the factors considered. The coefficients of multiple correlation (R) are presented in table 3, $\overline{R}$ giving the most probable value for the universe from which the sample was drawn. The coefficient of multiple correlation for date of first egg and date of last egg with egg production has about the same magnitude as the coefficient of simple correlation for length of laying year and egg production. Likewise, when length of laying year is used instead of date of first egg and date of last egg with other variables, the coefficients of multiple correlation are about the same as when the date of first egg and date of last egg are used. Evidently, then, as already pointed out, the influence of length of laying year on the egg production of turkeys is simply the combined influence of date of first egg and date of last egg, the two independent variables that determine the length of the laying year.

TABLE 3.—*Coefficients of multiple correlation*

Egg production correlated with—	1934		1935		1936	
	R	$\overline{R}$	R	$\overline{R}$	R	$\overline{R}$
Date of first egg; date of last egg	0.756	0.750	0.752	0.744	0.683	0.671
Date of first egg; length of pauses	.511	.494	.595	.580	.475	.451
Date of first egg; spring rate	.528	.512	.696	.686	.486	.462
Date of last egg; length of pauses	.884	.881	.882	.879	.897	.894
Date of last egg; spring rate	.672	.663	.716	.706	.610	.595
Length of pauses; spring rate	.356	.327	.477	.454	.518	.497
Date of first egg; date of last egg; length of pauses	.953	.951	.928	.925	.918	.914
Date of first egg; date of last egg; spring rate	.773	.764	.837	.829	.720	.705
Date of first egg; langth of pauses; spring rate	.585	.574	.726	.712	.598	.575
Date of last egg; length of pauses; spring rate	.900	.897	.894	.889	.907	.903
Date of first egg; date of last egg; length of pauses; spring rate	.970	.969	.953	.950	.959	.957
Length of laying year; length of pauses	.934	.932	.900	.807	.896	.893
Length of laying year; spring rate	.733	.725	.820	.814	.732	.723
Length of laying year; length of pauses, spring rate	.954	.952	.943	.940	.970	.969

In general, when egg production is correlated with two variables, the coefficient of multiple correlation so obtained is higher than the coefficient of simple correlation for egg production and either one of these variables (tables 2 and 3). When egg production is correlated with three variables, the coefficients of multiple correlation are still higher; and when egg production is correlated with all four variables, the highest coefficient of multiple correlation is obtained. The magnitude of the coefficient of multiple correlation obtained when egg production is correlated with all four variables is so close to 1 as to indicate that they account for substantially all the variance in first-year egg production in these groups of turkeys.

The relative importance of the four variables that have been shown to influence egg production can, as pointed out by Lerner and Taylor (7), be determined in several ways. The one adopted here is the coefficient of partial determination that measures the reduction in variance, due to the added factor, after the effects of the other independent factors have been accounted for (table 4).

TABLE 4.—*Reduction in unexplained variance, for factors considered, 1934–36*

Egg production correlated with—	Factors already considered	1934	1935	1936
Date of first egg	Date of last egg; length of pauses	0.571	0.366	0.180
Date of last egg	Date of first egg; length of pauses	.873	.783	.793
Length of pauses	Date of first egg; date of last egg	.781	.677	.701
Date of first egg	Date of last egg; spring rate	.257	.376	.221
Date of last egg	Date of first egg; spring rate	.436	.408	.361
Spring rate	Date of first egg; date of last egg	.048	.298	.085
Date of last egg	Length of pauses; spring rate	.782	.736	.755
Length of pauses	Date of last egg; spring rate	.652	.582	.714
Spring rate	Date of last egg; length of pauses	.119	.075	.081
Date of first egg	Length of pauses; spring rate	.249	.379	.111
Length of pauses	Date of first egg; spring rate	.091	.068	.160
Spring rate	Date of first egg; length of pauses	.112	.258	.149
Date of first egg	Date of last egg; length of pauses; spring rate	.670	.553	.544
Date of last egg	Date of first egg; length of pauses; spring rate	.909	.801	.874
Length of pauses	Date of first egg; date of last egg; spring rate	.853	.816	.833
Spring rate	Date of first egg; date of last egg; length of pauses	.365	.319	.489

The values obtained in the different years differ somewhat just as did those presented in tables 2 and 3. The order of the magnitudes is in good agreement for all 3 years. The data presented indicate that the relative importance of the influence of the four variables considered in determining first-year egg production is in the following order: Date of last egg, length of pauses, date of first egg, and spring rate. Date of last egg appears to be definitely the most important single factor determining egg production, whereas rate appears to be of least relative importance. Net spring rate does, however, exert a significant, if small, influence on egg production. Although length of pause is correlated with date of last egg, each of these factors exerts an influence on egg production apart from that of the others. (See tables 3 and 4.)

DISCUSSION

The data presented in this paper agree with those available for chickens. This agreement points to the conclusion that the internal factors influencing egg production are similar. Egg production in turkeys may therefore be regarded as a complex of characters that are probably influenced by a number of genes just as in the domestic fowl.

As has been pointed out above, the time factors (date of first egg, pauses, and date of last egg) have a greater influence on egg production than the rate factor. One might infer that the factors governing egg production in turkeys differ from those governing egg production in chickens, except that Lerner and Taylor (*7*) have obtained similar results with nonpausing populations of Single-Comb White Leghorns. Though there may prove to be some significant differences in sexual maturity in turkeys and chickens, the data presented in this paper as well as those of Marsden (*8*) and others indicate that the resemblances are much greater than the differences.

The difference in the best measure of sexual maturity (age at first egg in chickens, date of first egg in turkeys) is significant. It points to a greater influence of environment on egg production among turkeys than among chickens. The case has been stated in a different way by Scott and Payne (*10*), who point out that the reproductive cycle of turkeys resembles more closely that of birds in the wild state than it does that of other domesticated farm birds. Scott and Payne

found that housing, and factors in the environment other than light, had no influence on the egg production of turkeys. The implied difference in the sexual maturity of chickens and turkeys, therefore, probably does not result from the fact that the turkeys were not housed and that chickens invariably are.

Though persistency (date or age at last egg) has the greatest influence on annual egg production in turkeys, other factors may be of equal importance because of their influence on egg production during the breeding season. Further data are needed to determine whether such is the case.

SUMMARY

A statistical analysis was made of the first-year records of 238 turkeys which were trap-nested in 1934, 1935, and 1936 and which had in each case been hatched in April and May of the preceding year.

Date of hatch was not found to have any significant, consistent influence on egg production.

Date of first egg was more closely correlated with egg production than was age at first egg. Date of first egg was accordingly selected as the more suitable measure of sexual maturity.

Date of (and age at) last egg was more closely correlated with egg production than was any other single factor. Date of first egg was not correlated with date of last egg.

Length of laying year was correlated with egg production, the zero order coefficients having about the same magnitude as the coefficients of multiple correlation for egg production and the two variables (date of first and date of last egg) that determine length of laying year. The coefficient of multiple correlation for length of laying year with date of first egg and date of last egg ranged from 0.976 to 1.000.

Total length (but not number) of pauses was correlated with egg production. Length of pause was correlated with date of last egg, but not with date of first egg nor with spring rate.

Net spring (March and April) rate was correlated with egg production, whereas annual net rate was not. Spring rate was not correlated with date of first egg, date of last egg, or length of pauses.

The coefficients of multiple correlation of egg production with date of first egg, date of last egg, length of pauses, and net spring rate approached the maximum possible value, varying from 0.950 to 0.970, a fact indicating that these four factors determine most, if not all, of the variance in first-year egg production.

The four factors found to influence annual egg production, particularly as measured by the coefficient of partial determination, are, in order of decreasing importance: (1) Date of last egg (persistency), (2) length of pause, (3) date of first egg, (4) net spring rate.

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